POLYCYCLIC AROMATIC HYDROCARBONS IN SELECTED FISHES FROM THE ATHABASCA AND SLAVE RIVERS, CANADA

A Thesis Submitted to the College of

Graduate Studies and Research

In Partial Fulfillment of the Requirements

For the Degree of Doctor of Philosophy

In the School of Environment and Sustainability

University of Saskatchewan

Saskatoon

Canada

By

Ehimai Ohiozebau

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ABSTRACT

Human activities over the years, especially the unconventional exploitation of oil sands deposits, downstream on the Athabasca River (AR), might have affected the water quality and ecological integrity of the river basin, thereby presenting a threat to the environment and human health. There have been concerns that the oil sands process-affected waters stored in tailing ponds may be percolating to surface waters as well as underground waters, contaminating neighboring watersheds with a cocktail of chemicals including Polycyclic aromatic hydrocarbons (PAHs). PAHs are present both naturally and from human activities as pollutants in the environment. Forest fires, geologic activities, and oil seeps are examples of natural sources of PAHs in the environment. The major sources of PAHs in the Athabasca region are leaching of oil sands deposits and contamination from oil sands production. On occasions, forest fires contribute PAHs in the area. There has been no comparative data on the exposure of PAHs to fish along the AR and Slave River. I used an integrative monitoring of selected fishes as an indicator to achieve four objectives: i) describe the spatial and seasonal distribution of measurable concentrations of products of biotransformation of polycyclic aromatic hydrocarbons (PBPAH) in bile of fish; ii) determine the levels of parent PAHs in the muscle of fish, and extrapolate the data to estimate potential risk to human consumers, and to identify which species and geographic regions, if any, pose the greatest risk to humans; iii) use patterns of contamination to provide a scientific basis for elucidating the source of contamination; and iv) perform fish health investigation by collecting morphometric health measures and perform a systematic assessment of the occurrence of lesions in the fishes. I sampled whitefish (*Coregonus clupeaformis*), jackfish/northern pike (*Esox luscius*), walleye (*Sander vitreus*), goldeye (*Hiodon alosoides*) and burbot (*Lota lota*) from Fort McMurray, Fort McKay, and Fort Chipewyan in

Alberta, and from Fort Smith and Fort Resolution on the Slave River in the Northwest Territories. The rationale for selecting fishes included: their abundance along the basin (some have short ranges, e.g., northern pike); their dietary/nutritional and cultural significance to communities in the area; their feeding strategy, such as benthic, supra-benthic, or pelagic, trophic status, and patterns of migration and habits of spawning. I addressed the first objective in Chapter 2, where the total PBPAHs were determined. Concentrations of products of biotransformation of 2 and 3-ringed, 4-ringed, and 5-ringed PAHs were measured using synchronous fluorescence spectroscopy. Spatial and seasonal differences were observed with greater concentrations of PBPAHs in samples of bile of fish collected from Fort McKay as well as greater concentrations of PBPAHs in bile of fish collected during summer compared to those collected in other seasons. Overall, PBPAHs were greater in fishes of lower trophic levels and fishes more closely associated with sediments. In particular, goldeye (*Hiodon alosoides*), consistently contained greater concentrations of all the PBPAHs studied. In Chapter 3, I achieved the second objective by measuring levels of parent PAHs in muscle of selected fishes and extrapolated the results to determine potential human health risks due to fish consumption. Dorsal muscle of fishes from upstream reaches of the AR close to oil sands extraction and upgrading activities, contained greater concentrations of individual PAHs than concentrations in muscle of fishes from further downstream in the Slave River. Risks posed by PAHs to humans were assessed using a B[a]P equivalents approach. According to the risk assessment results, the average lifetime risk of additional cancers for humans who consumed fish was less than 10^{-6}. In Chapter 4, alkylated PAHs were also measured in fish muscle to achieve the third objective. The general presence of naphthalenes and phenanthrenes and the evaluation of molecular ratios (i.e., LMW/HMW alkyl-PAHs) allowed me to conclude that the major source of pollution is

petrogenic, probably due to increases in oil sand activities around Fort McMurray and Fort McKay. I achieved the fourth objective in Chapter 5 by studying the health status and potential effects of industrial development on individuals of economically and culturally significant fishes. A resurgence in condition factor of all species after a low in 2011 was observed. Annual variation was also observed in condition factor and the incidence of anomalies or lesions. Morphometric data demonstrated relatively consistent health among fishes in both the Athabasca and Slave rivers. Analysis of condition factor and somatic indices did not demonstrate consistent differences along the river system. Overall, the health of fish as determined by the metrics employed in this study, does not appear to be adversely affected by the current level of development in the Alberta oil sands region. The data presented in this dissertation make invaluable contribution to the much needed monitoring program in the Athabasca and Slave Rivers. Overall, my findings provide baseline data on fish health, concentrations of parent and alkylated PAHs, and products of biotransformation of PAH in five species of large-bodied fishes consumed by humans in communities in the Lower Athabasca and Slave River basin. These results will be useful for establishing the status and trends and spatial distribution of PAHs during monitoring of the lower Athabasca basin and most importantly, as a valuable reference point before any potential permitted discharges of wastewaters from processing of oil sands to the AR.

ACKNOWLEDGMENTS

I am very grateful to have had the opportunity to complete my graduate studies under the supervision of Dr. Paul D. Jones. He served as a teacher and mentor throughout my PhD program. I thank Dr. John P. Giesy, Dr. Garry Codling and Brett Tendler for their help and guidance every step of the way. I would like to thank members of my advisory committee: Dr. Markus Hecker, Dr. Christy Morrissey, Dr. Lee Wilson, and Dr. Doug Clark for their continued support. I was blessed to have Dr. Derek Muir serve as the external examiner for my defence, and I thank him for his flexibility and insightful comments. I would like to thank my wife, Diana Ohiozebau, my daughter, Aye Ohiozebau, friends and family for their love and support. And finally, I would like to thank everyone in Toxicology Centre and the School of Environment and Sustainability.

DEDICATION

This thesis is dedicated to the memory of my father, Hon. Ohiozebau P.A. Aigbevboile (1940–2011) who made key contributions to my education.

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List of Abbreviations

Alky	Alkylated
ANOVA	Analysis of Variance
AR	AR
AOSR	Athabasca Oil Sand Area
BaPeq	Benzo(a)pyrene Equivalent
CF	Condition Factor
DCM	Dicloromethane
DI	Daily Intake
EEM	Environmental Effect Monitoring
FAC	Fluorescently Active Compounds
FC	Fort Chipewyan
FF	Fort Fitzgerald
FM	Fort McKay
FMU	Fort McMurray
FR	Fort Resolution
FS	Fort Smith
GC/MS	Gas Chromatography/ Mass Spectrometry
GSI	Gonad Somatic Effect
HCA	Hierarchical Cluster Analysis
HMW	High Molecular Weight
HPLC	High Performance Liquid Chromatography
HSI	Hepatoma Somatic Index

ILCR	Incremental Lifetime Cancer Rates
LMW	Low Molecular Weight
LOD	Limit of Detection
LSI	Liver Somatic Effect
NA	Naphthenic Acid
OSPW	Oil Sand Process-affected Water
PACs	Polycyclic Aromatic Compounds
PAHs	Polycyclic Aromatic Hydrocarbons
PCA	Principal Component Analysis
PP	Peace Point
PEF	Potency Equivalent Function
PDF	Probability Density Function
PBPAHs	Products of Biotransformation of PAHs
QA	Quality Assurance
QC	Quality Control
SD	Standard Deviation
SFS	Synchronous Fluorescence Spectroscopy
SIM	Selected Ion Monitoring
SR	Slave River
TEF	Toxic Equivalent Factor
USEPA	United States Environmental Protection Agency
WM	Wet Mass

Chapter 1: General Introduction

1.1 Polycyclic Aromatic Hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are a group of organic chemicals that are ubiquitously present as pollutants in the environment (Lin et al., 1994). They are made up of two or more condensed aromatic rings with molecules containing only carbon and hydrogen atoms (Angerer et al., 1997). PAHs are present both naturally and from anthropogenic activities as pollutants in the environment. The most significant anthropogenic sources are pyrogenic and petrogenic. Examples of ways that PAHs can be released are production of petroleum coke and charcoal, burning of fossil fuels and wood, cooking at high temperatures, and cigarette smoking. Petrogenic PAHs are mainly from petroleum refining, and petroleum spills (Pampanin and Sydnes, 2013). Pyrogenic PAHs have greater proportions of unsubstituted parent compounds while petrogenic PAHs are dominated by alkylated forms of the parent homologues (Akre et al., 2004). Incomplete combustion processes and petrochemical pollution are the main causes of aquatic PAH pollution (Boehm et al., 2007; Peters et al., 2005).

The adverse biological effects associated with PAH contamination have been studied and described (Eichbaum et al., 2014; Lee et al., 2015; Lin et al., 2015). PAHs may cause cancer but they require metabolic activation to exert their carcinogenic effects (Angerer et al.,1997). Some PAHs are classified as carcinogenic because they are metabolized to intermediates (e.g., dihydrodiols) by hydrocarbon hydroxylases present in the liver. PAHs with four or more condensed benzene rings are often mutagenic and/or carcinogenic (Johnson et al., 2004). Toxicity is mainly dependent on the chemical structure, isomers with the same number of rings may vary from non-toxic to extremely toxic depending on the different steric positioning of the benzene rings (Fig 1.1) (Angerer et al., 1997; Huang et al., 2012). The structures of products of biotransformation of PAHs with epoxide in bay regions and fjord regions or sterically hindered

bay region are likely to be potent carcinogens (Fig 1.2). These intermediates can then form adducts with DNA (Baird et al., 2005; Khan et al., 1999). As in mammals, dihydrodiols and their epoxide derivatives covalently bind between bases of DNA and proteins in fish, starting mutagenic processes in the cell (Lin et al., 2015).

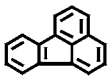
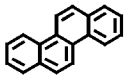
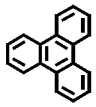
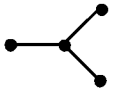
Compound	Formula	Graph	Characteristics
	Peri-Condensed		
Pyrene			cycle alternant
Fluoranthene			cycle non-alternant
	Cata-Condensed		
Chrysene			linear non-branched alternant
Triphenylene			linear branched alternant

Figure 1.1: Different types of PAH structures. Peri-condensed PAHs can be defined as those whose lines connect the ring centers, and form cycles. Cata-condensed PAHs can be defined as those systems whose lines do not form cycles, and can be classified as branched or not branched (Guillen and Sopelana 2003).

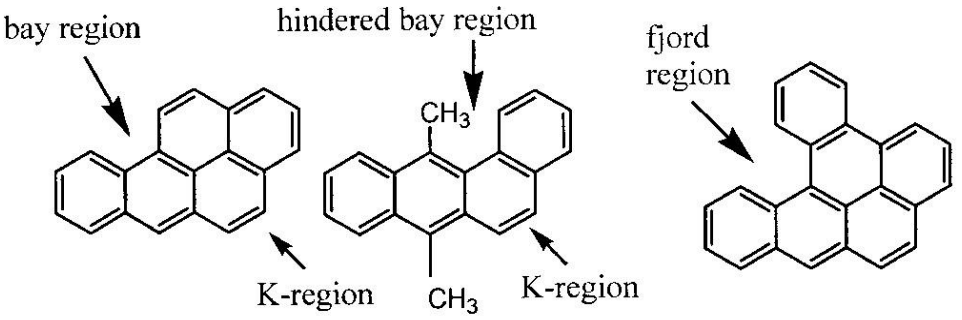


Figure 1.2: Structural features of PAHs that contribute to carcinogenicity. B[a]P, a known carcinogen, contains a bay region. The diol epoxide metabolite of B[a]P, which binds to DNA, contains an epoxide group next to the bay region. Methyl groups in DMBA hinder this structural feature further, as does the extra benzene ring in DB[a,l]P. The compounds are arranged in order of their increasing carcinogenic potency, with DB[a,l]P being the most potent (Bird et al., 2005).

Many studies have shown that high PAH concentration are toxic to fish and other aquatic organisms (Adams et al., 2014; Bauder et al., 2005; Lin et al., 2015). During phase 1 metabolism, PAHs are biotransformed in fish to epoxides, phenols, quinones. To enable excretion, they are further converted in phase 2 metabolism into a highly water-soluble conjugates (e.g., glucuronides, sulfate, or glutathione conjugates). Phase 1 and phase 2 biotransformation of PAHs take place in the liver, and the gall bladder serves as a storage site for metabolic products. However, because of enterohepatic circulation, some of these metabolites within the organism have more time to exert adverse toxic actions before they are finally eliminated.

In aquatic environments, PAHs are mainly associated with biota, sediments and suspended particles (Walker et al., 2012). However, sediment concentrations may not be the best indicator for environmental studies because of heterogeneity of contaminants in sediments and differences in contaminant bioavailability. There are many factors that influence the bioavailability of PAHs including ingestion of suspended matter, solubility of the particular PAH compound and the preferred habitat and food source of the species in question (Borga et al., 2004). Exposure of PAHs in fish is commonly used in aquatic pollution assessment programs (Dû-Lacoste, 2013; Lin et al., 1996). Exposure can be addressed by measuring contaminant levels in muscles or their metabolites in the bile of aquatic organisms (Lin et al., 1994). Biotransformation, excretion and relative rates of uptake of PAHs largely inform the feasibility of using bile and tissue concentrations for exposure studies (EscartÍn & Porte 1999; Fuentes-Rios et al., 2005; Gibbons et al., 1995).

In the Athabasca area, PAHs have been detected in many organisms (Colavecchia et al., 2004; Fleeger et al., 2007) including aquatic insects (Wayland et al., 2008), and low levels in water-bird eggs (Hebert et al., 2011). This has raised concerns about the potential environmental effects on aquatic ecosystems, especially on some fish species which are mostly eaten as country food by First Nations' communities living in northern Alberta and the Northwest Territories. Some residents of downstream communities, especially in Fort Chipewyan on the Peace-Athabasca delta have expressed concern that oil sands development is contaminating country foods by contributing higher than natural levels of PAHs to the environment (Timoney and Lee, 2009; Chen, 2009). Possible contamination of fish and aquatic wildlife with PAHs in reclaimed oil sands lakes and wetlands has also been reported (Van den Heuvel et al., 1999; Smits et al., 2000; Gurney et al., 2005).

1.2 Oil sands

The Canadian Oil Sands deposits are usually found below 75m of soil surface, but where the Athabasca River (AR) and its tributaries have incised into this formation, surface exposure is often observed (RAMP, 2009; Timoney, 2007; Wayland et al., 2008). As a result of the near surface formation, the exploitation of oil sands is different from conventional petroleum extraction processes (Conly et al., 2002). The oil sand deposits are surface mined using huge mobile diggers to remove surface earth that is piled up to forms hills in operations areas. Between 60 to 70 meters deep, oil sands are harvested in large chunks. The method pioneered by K.A. Clark in the 1920's is used to separate oil from ore. The bitumen extraction method requires considerable amounts of hot water and caustic soda. About 2-5 barrel of water is used for the alkaline hot water extraction of one barrel of oil (Dowdeswell et al., 2010; Giesy et al., 2010). The extraction process leaves waste slurry of different components containing oil sands process-affected waters (OSPW), sand, clay and residual hydrocarbons and is known as oil sands tailings. These tailings are stored on site in large settling ponds. About $4m^3$ volume of slurry waste is generated for each cubic meter of mined oil sands (Holowenko et al., 2001; Headley and McMartin, 2004). OSPW contains a cocktail of chemicals including residual bitumen, PAHs, benzothiophenes, dibenzothiophenes, and naphthenic acids (Clemente et al., 2003). Many of the chemicals in OSPW are harmful to the environment (Debenest et al., 2012; He et al., 2012).

Currently, oil sands companies operate under a zero discharge policy. Alberta's provincial legislation prohibits the release of toxic waste in any form including OSPW to the environment. Much of the water is expected to be recycled through the extraction process and waste stored in tailing ponds (Clemente and Fedorak, 2005). Before dewatering tailing ponds for reclamation, the waste is expected to be converted to 'pristine' before release into surrounding surface water.

It is also required that the disturbed land be reclaimed at some point in time. However, Suncor is allowed to discharge some of their cooling water, process effluent, and surface runoff waters from their upgrader site. To what extent the reclaimed ponds are restored to "equivalent land capability" is somewhat contentious.

1.3 Source and Extent of Contamination

Despite the statutory zero discharge policy, concerns have been raised over the potential downstream environmental contamination from tar sand operations. The concerns expressed in some quarters have been centered on the unintentional release of stored OSPW and general pollution from oil sands operations (Timoney and Lee, 2009). Possible sources of pollution from oil sands operations includes fugitive dusts, tailing piles, burning of natural gas to generate steam needed for bitumen extraction, gas flaring, stationary fuel combustion, industrial process venting, establishment dust and runoffs from mines, and general land disturbance (Dillon et al., 2011). It is also possible for oil sand process affected waters (OSPW) stored in the tailing ponds to:

- Percolate and contaminate underground waters, and adjacent surface waters;
- Accidentally discharge and spill to nearby surface water;
- Act as death traps to migratory birds (Timoney and Lee, 2009);
- Release air borne contaminants;
- Cumulatively stress aquifers, surface and ground water (Jordaan, 2012); and
- Cause acidification from air emission.

The source and extent of aquatic environmental contamination from oil sands operations in northern Alberta is largely unknown and controversial. The uncertainty is due to the unique terrain of oil the sands formation (natural incision which makes it difficult to differentiate natural

from anthropogenic chemical sources), and the limited number of baseline studies before tar sands operations were started. Monitoring programs were put in place after oil sands operations began (Dillon et al., 2011). The few baseline studies available focused on fluvial sediments of the AR and the depositional environment of Peace Athabasca Delta (Conly et al., 2002). Source identification is further complicated by the different reports of chemicals released to the environment from oil sands operations. The subject has led to considerable controversy in academia with different peer reviewed papers arriving at opposite conclusions (Hall et al., 2012; Wiklund et al., 2012; Kelly et al., 2010; Kelly et al., 2009). Several groups (Kelly et al., 2009; Kelly et al., 2010; Timoney and Lee, 2009; Gagné et al., 2011) report that oil sands development contributes PAHs and heavy metals (e.g. Hg & As) to the AR and its tributaries, while Hall et al., (2012) and Wiklund et al., (2012) argued differently. The mixed empirical results have made the sources of PAHs in the AR unclear.

Kelly et al., (2009) conducted a study to determine if oil sands operations contribute polycyclic aromatic compounds (PAC) to the AR and its tributaries. Atmospheric depositional loadings of PAHs were measured in snowpack. PAHs were also measured in samples from the AR and its tributaries. Sites were selected upstream and downstream of oil sands operations, along the AR. Increases in the deposition of PACs were observed in snowpack close to Suncor and Syncrude mining and processing facilities. The authors suggested that patterns of PACs in melted snow collected close to mining facilities emanated from oil sands operations' particulate emissions. They concluded that oil sands development released greater amounts of PACs than previously recognized. The finding is consistent with other findings (Hsu et al., 2015; Timoney and Lee, 2009 & 2011). However, Hall et al., (2012) used a paleo-hydrological approach with sediment cores to understand trends in river transported hydrocarbon associated contaminants.

They argued that natural processes (like increase in flood frequency and wind erosion) were the major source of PAHs in the Peace Athabasca Delta. They further argued uncertainty in temporal measurement, sediment remobilization, and different sampling methods as factors that make source discrimination of PAHs in the AR and Delta difficult.

1.4 Athabasca and Slave Rivers

Most of the operational sites of oil sand industries in northern Alberta are located near the AR that is part of the Athabasca basin. Streams, rivers, lakes, delta, and wetlands are components of the Athabasca basin, which originates as the AR from melting glaciers in the Columbia Ice Fields in the Rocky Mountains. The AR joins the Peace River northwards to form the Slave River (SR) that empties into the Great Slave Lake, part of the Mackenzie River systems and eventually flows into the Arctic Ocean, after receiving waters from about 157, 000 km^2 of drainage area (Squires et al., 2010). The AR flows through Jasper, Hinton, Whitecourt and Fort McMurray through the Peace Athabasca Delta and into Lake Athabasca south of Fort Chipewyan. Along the way a system of tributaries including the Clearwater River, MacKay River, Muskeg River, Embarras River, Fletcher Channel and numerous smaller tributaries join the AR.

Parts of the AR are naturally incised into the oil sand formation. This makes the explicit identification of the oil source(s) a challenge. To assess and predict potential impacts of industrial activities in the AR basin it is important to separate these impacts from those produced by naturally occurring hydrocarbon deposits and releases (Headley and Akre, 2001). Possible contamination of fish and aquatic wildlife by PAHs in reclaimed oil sands lakes and wetlands has been reported (Van den Heuvel et al., 1999; Smits et al., 2000; Gurney et al., 2005) but no

study has simultaneously attempted to compare various fish samples from Forts McMurray, Mckay, Chipewyan, Smith and Resolution over time.

1.5 Monitoring of Polycyclic Aromatic Hydrocarbons

It is difficult to decipher the relative natural load versus industrial contributions of PAHs to the environment. The difficulty is mainly as a result of the continuous inflow of natural hydrocarbons from incised and outcropped oil sands deposits. Some of these incisions and eroded deposits are in the same area where surface mining and processing is taking place.

Attempts have been made to differentiate the types and ratios of hydrocarbon contaminants that are associated with natural incision from those associated with oil sands operations (Headley et al., 2001; Akre et al., 2004; Conly et al., 2002). Sources of conventional oil are often established by double ratio plots, analysis of hopane biomarkers, principle component analysis, and comparison of alkylated PAH fingerprints (Akre et al., 2004). Monitoring of PAHs in the Athabasca showed a general trend towards greater proportions of alkylated PAHs compared to parent PAHs (Akre et al., 2004). Baseline researches have been carried out to characterize the natural source contributions of oil sands material entering the river system by studying weathering ratios (Strosher and Peake, 1979). Petroleum released into the environment is subject to weathering which is a range of chemical, physical, and biological processes that change the composition of hydrocarbon mixtures released to the environment (Bence and Burns, 1995). There has been progress in providing baseline information for water, sediments and air (Ross et al., 2012; Zhang et al., 2016). Conly et al., (2002) and Prince et al., (2002) independently tried to establish the weathering patterns in the AR by studying the photochemical decomposition of spilled oil and Akre et al., (2004) established biodegradation as the major pathway.

Chemical fingerprinting of a PAH mixture can also lead to the identification of the specific contamination source. Sauer et al., (1993; 1994) reported the detailed use of biomarkers for investigations of source oil identification and oil migration. The source of oil is often also established by the comparison of alkylated PAH fingerprints: i.e., extensive alkylation points to a predominantly petrogenic contamination source and less alkylation indicates a predominantly pyrogenic source (Sauer et al., 1993; Wang et al., 1999). Other studies have used previous data (e.g., 1999 to 2009) as baseline condition (Timoney and Lee, 2009). When oil sands operation ends, toxic components in OSPW are expected to be reduced to below acceptable benchmarks, and disturbed land reclaimed. With the current volume of contaminated water stored in tailing ponds, there is doubt about the possibility of producing water of 'acceptable' quality before eventual discharge into the AR system (Kean, 2009; Rooney et al., 2012; Fleming et al., 2012). Baseline information would help our understanding of natural versus anthropogenic contributions to bitumen related contaminants in the Athabasca region.

Despite the uncertainty and controversy surrounding the actual chemical source(s), there is understanding that bitumen related contaminants are present in the environment of the Athabasca Oil Sand Region (Dillion et al., 2011) but it is unclear if and how the contaminants directly affect human health. The uncertainty in human health risk is a result of significant gaps in risk assessments, health and environmental data, and lack of information transparency (Weinhold, 2011).

1.6 Study Locations

Figure 1.3 shows the study locations. Fort McMurray ($56^0$43'35" N $111^0$22'49" W) is located near the oil sands in the Regional Municipality of Wood Buffalo in Alberta, Canada. It is

nested in a forest valley where the Athabasca and Clearwater rivers meet. It has a population of about 61, 374 (Statistics Canada, 2011) from all over Canada and the world. Fort McMurray is best known for its association with the oil sands industry. Besides the oil sands, the economy also relies on natural gas and oil pipelines, forestry and tourism. Fort McKay (57^0N 112^0W) is located along the banks of the AR in the Regional Municipality of Wood Buffalo, approximately 54 km north of Fort McMurray with population of 562 (Statistics Canada, 2007), predominantly Dene, Cree and Métis community. Like Fort McMurray, the economy is centered on the development of the Athabasca oil sands to the immediate south and north. The hamlet of Fort Chipewyan (59^0N 111^0W) in northern Alberta is one of the most northern communities of the Regional Municipality of Wood Buffalo. It is located on the western end of Lake Athabasca, adjacent to Wood Buffalo National Park with a population of 847 (Statistics Canada, 2007). Fort Chipewyan has a land area of 10.23 km^2 with a population density of 82.80/km^2, predominantly made up of Cree, Chipewyan (Dene), and Métis people. Fort Smith (60º00'19" N 111º53'26" W) is located in the southeastern portion of the Northwest Territories, nestled in the boreal forest along the banks of the SR and adjacent to the NWT/Alberta border with a population of about 2,500 (Statistics Canada, 2011). Fort Smith is a multicultural community with the population being majority First Nations and Métis. Fort Resolution ($61^0$10'18" N $113^0$40'18" W) is a remote coastal community with approximately 95% of its residents identifying as Dene and Métis (Statistics Canada, 2007). It is situated on the south shore of Great Slave Lake.

Many residents of Fort Chipewyan, Fort Smith and Fort Resolution have a close connection and reliance on the local ecosystem for sustenance, similar to other Aboriginal groups in northern Canada (Cunsolo-Willox et al., 2012; Parlee et al., 2005, 2006). In addition, some people own or work for local small businesses. Considering the remote location of the

communities and the higher cost of living in the north, staple food items such as milk and meat cost two to three times more than in other Canadian communities (Statistics Canada, 2006). Like other northern communities, the practice of hunting, trapping, and fishing are required for nutrition and subsistence (Macdonald et al., 2013; Parlee et al., 2012). While the composition of country foods varies between the communities, foods mainly consumed include a number of species of berries, rabbit, caribou, duck, moose, goose, and fish, among others (Parlee et al., 2012). The lasting environmental effects of oil sands extraction has potential to be compounded by the socioeconomic and health changes that can occur to residents (Westman, 2006). The mining and processing of oil sands can spread toxic concentrations of PAHs and other petroleum related contaminants over large areas of surrounding land and water (Kelly et al., 2009). The emissions have potential to significantly contaminate nearby country food, which serves for the subsistence, nutrition, and economic well-being of residents. For residents, whose livelihoods and cultural practices are often tied closely to the local environment, these long-lasting environmental effects may be devastating (Nuttal, 2006; Westman, 2006).

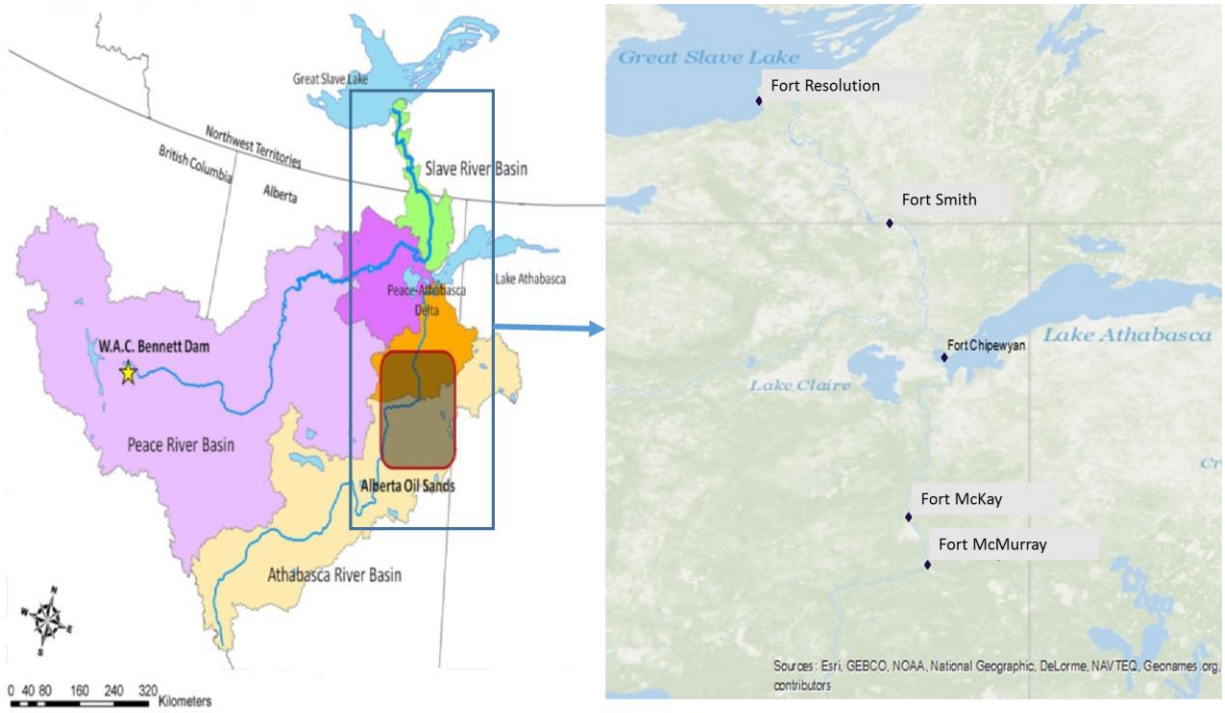


Figure 1.3: Map of the Athabasca and Slave Rivers. The Alberta Oil Sands and Sampling Locations are highlighted.

1.7 Research Objectives

The research detailed in the chapters of this thesis endeavors to describe the spatial and seasonal distribution of PAHs in bile and muscle of selected fish species.

The specific research objectives and evaluated hypotheses tested were as follows:

Objective 1: Determination of the spatial and seasonal distribution of measurable concentrations of products of biotransformation of polycyclic aromatic hydrocarbons (PBPAH) in bile of selected fish.

Ho1: There is no spatial and seasonal distribution of measurable concentrations of products of biotransformation of polycyclic aromatic hydrocarbons (PBPAH) in bile of selected fish.

Objective 2: Determination of the levels of parent PAHs in the muscle of fish, and extrapolate the data to estimate potential risk to human consumers, and to identify which species and geographic regions, if any, pose the greatest risk to humans.

Ho2: There is no potential risk to humans who frequently consume fishes from the Athabasca and Slave Rivers.

Objective 3: use patterns of contamination to provide a scientific basis for elucidating the source of polycyclic aromatic hydrocarbons in the Athabasca and Slave Rivers

Ho3: There is no petrogenic or pyrogenic source of PAHs in the Athabasca and Slave Rivers.

Objective 4: perform fish health investigation by collecting morphometric health measures and perform a systematic assessment of the occurrence of lesions in the fishes.

Ho4: Stressors in the area do not cause health effects on selected fishes in the Athabasca and Slave Rivers.

1.8 Thesis Structure

This thesis is presented in the 'dissertation by manuscript' style and follows the guidelines set out by the College of Graduate Studies and Research. Following this introductory chapter, the thesis is organized into four manuscripts, each of which is presented as a single thesis chapter.

Chapter 1 provides background information of this study including oil sands, Athabasca and Slave Rivers, source and extent of PAH contamination, monitoring of PAHs, human health risks, and analytical techniques. In Chapter 2, I focused on the products of biotransformation of

polycyclic aromatic hydrocarbons (PBPAHs) in fishes of the Athabasca and Slave River System, Canada. There, I characterized PBPAHs in the bile of selected fishes, determined spatial and seasonal distribution of measurable concentrations of PBPAHs in bile of fishes, and determined the species with the greatest exposure to PBPAHs. Chapter 3 dwells on the potential health risks posed by polycyclic aromatic hydrocarbons in muscle tissues. The nature and extent of potential exposures to people who frequently consume the selected fishes were also evaluated. Furthermore, I identified which species and geographical regions pose the greatest risk and also identify the population group that is likely the most susceptible. The spatial and seasonal distribution of measurable concentrations of parent PAHs were determined. Chapter 4 focuses on pattern recognition of alkyl-PAHs in muscles of fishes from the Athabasca and Slave Rivers, Canada. It determines the spatial and seasonal distribution of measurable concentrations of alkylated polycyclic aromatic hydrocarbons, used the degree of alkylation to elucidate the potential source(s) of PAHs, and Identified the geographical region with the greatest degree of alkylation. In chapter 5, I focused on health status of fishes from the Athabasca and Slave Rivers, Northern Canada. I utilized the morphometric data to determine the health status fishes of nutritional, commercial and recreational significance to residents, determined specific measures of gross external and internal anomalies, and evaluated relative incidences of anomalies among locations. Finally, in chapter 6, I gave a synopsis of my research, reported key findings, and suggested areas requiring further research.

1.9 Sample Collection

Fishes were collected, in cooperation with First Nations fishers, and regional and federal agencies, from five locations along the Athabasca and Slave Rivers (Fig. 1.3). Frequency of

sampling was seasonal: June/July (summer), October (fall) in 2011 and May (spring) 2012. Species collected were; whitefish (*Coregonus clupeaformis*), jackfish/northern pike (*Esox luscius*), walleye (*Sander vitreus*), goldeye (*Hiodon alosoides*) and burbot (*Lota lota*). Locations were close to: Fort McMurray ($56^0$43'35" N $111^0$22'49" W), Fort McKay (57^0N 112^0W), Fort Chipewyan (59^0N 111^0W), Fort Smith ($60^0$00'19" N $111^0$53'26" W) and Fort Resolution ($61^0$10'18" N $113^0$40'18" W). Fort McMurray is upstream of the major oil sand operations on the AR; it is however within the McMurray Formation where natural incision of surface waters into the formation can liberate contaminants derived from bitumen.

Fishes were collected using gill nets (10.80 cm mesh), and were placed on ice for transport to the field laboratory to determine the biometrics, perform a systematic assessment of the occurrence of lesions, and to gather tissue samples and supporting health information. After euthanasia, each fish was measured, weighted, photographed and examined for the presence of external lesions, malformations and other signs of poor health. Fish were then opened ventrally from the vent to the pericardium and the left side of the body was removed to reveal the internal organs for examination. Heart, liver, stomach, intestine, kidney and spleen were examined, masses of liver, spleen and gonads were determined. Examinations were consistent with Canada's Environmental Effects Monitoring (EEM) procedures (www.ec.gc.ca/eem). The size of fish obtained was influenced by mesh size of the net (10.80 cm) used during collections. Fish caught during this study represent only a portion of the size and age spectrum of the population. After examination, the gall bladder was removed and immediately stored at -80 ^{0}C until characterization of PBPAHs by SFS. Muscle tissue samples were collected from the mid-body dorsal area and were frozen at -18°C in pre-cleaned amber jars for PAH analysis.

1.10 Human Health Risk Assessment

Risk estimation of PAH exposures is a complex issue. PAHs in the environment come from several sources and are several hundred different chemical congeners to consider. Individual PAHs may represent a cancer risk by more than one mechanism (Bostrom et al., 2002). This renders risk estimates very uncertain. However, as a result of the paucity of data and publications on human health risk assessment to PAHs and the present stage of knowledge, risk estimation for PAHs at low exposure levels are generally based on the assumption of a linear dose-response relationship (Bostrom et al., 2002; Peng et al., 2011).

To assess human exposure to carcinogenic PAHs from fish consumption, I used a probabilistic risk model. The probabilistic risk assessment framework used probability density functions (PDFs, e.g. fish consumption rate) available in Canada (Northwest Territories) for risk estimation. These PDFs were sourced from those defined by the most recently available and reliable data collected in Canada especially from peer reviewed journals and government agencies (Richardson, 2013). This model used here mainly focuses on the relationship between fish consumption and potential cancer incidences in the study areas. Collins et al., (1998) developed a potency equivalence factors (PEFs) scheme to estimate the sum carcinogenic potency of all considered PAHs while Chen and Liao, (2006) defined occupancy probability as the likelihood of the time a person spends in a specific place. The probabilistic risk model integrated the potency equivalence factors (PEFs), age group-specific probability and the incremental lifetime cancer risk (ILCR) to quantitatively estimate the temporal and spatial exposure risk for age groups of adults, children, and infants living in sampling areas.

1.11 Analytical Techniques

Various analytical methods have been developed for the extraction and analysis of PAHs from fish samples. Most of the analytical steps for the extraction of PAHs from fish samples consist of extraction, hydrolysis, purification, concentration, identification and quantification Liguori, (2006). The classical separation protocols of PAHs from fish tissues have mainly been saponification in basic alcoholic solutions. This process requires the presence of alcohol, which has been reported to interfere with the determination of alkylated PAH derivatives (Linguori et al., 2006). Yang et al., (2003), Aas et al., (2000) amongst others reported the use of fixed wavelength fluorescence (FF) for the preliminary determination of PAHs in fish bile. Hawkins et al., (2002) demonstrated the use of a more rapid non-specific synchronous fluorescence spectrometry (SFS) screening method for PAHs. SFS has been effective as a screening tool for urine of other aquatic organisms (Dissanayake and Galloway, 2004; Eickhoff et al., 2003). The SFS approach has also been utilized for other PAH detecting purposes, such as for screening of benzo[a]pyrene in foods with a high fat content (Garcia-Falcon et al., 2000), for identification of PAH exposure in tissue from marine polychaetes (Giessing et al., 2003; Tairova et al., 2009). Other analytical methods reported for the determination of PAH metabolites in bile are High Performance Liquid Chromatography (HPLC)-Fluorescence screening (Leonard and Hellou, 2001 and Stroomberg et al., 2003), and gas chromatography with mass spectrometry (MS) (Capotorti et al., 2004; Hornung et al., 2007, and Simpson et al., 2002).

Specifically, SFS was used to screen and fingerprint PAHs in fish bile. SFS assays of diluted fish bile often yield very characteristic fluorescence spectra that are dependent on the particular PAH metabolites present in the mixture. In SFS, the excitation/emission light detection conditions are varied simultaneously, with a fixed wavelength differential and over a specific

wavelength range (Aas et al., 2000). Also, GC/MS was used to determine the levels and parent and alkylated PAHs in the muscle of selected fish. Fish have high depuration rate and as a result, it is expected that the bioaccumulation of parent and alkylated PAHs will be low. However, there is need to analyze the muscle tissues of these fishes to determine the possible concentration of PAHs for risk assessment. In this study, 16 USEPA parent and alkylated PAHs were investigated in muscles tissues of sampled fish species. PAHs were identified and quantified using a Hewlett Packard (HP) 7890A GC fitted with a 60 m, 0.25 mm i.-d., DB-5 silica capillary column and an HP 7683 series autosampler. The alkyl-PAH analysis was also performed using an Agilent 7890A gas chromatograph interfaced to an Agilent 5975 series Mass Selective Detector and a 7683 series autosampler.

Specific QA/QC procedures, results, statistical analyses, number of individuals, and experimental methodologies are provided in each of the relevant experimental chapters.

Preface to Chapter 2: Products of Biotransformation of Polycyclic Aromatic Hydrocarbons in Fishes of the Athabasca/Slave River System, Canada

The first object of this thesis was to determine the spatial and seasonal distribution of measurable concentrations of PBPAH in bile of five fishes of nutritional, cultural and ecological relevance from the Athabasca/Slave River system. We collected samples in Alberta and the Northwest Territories, Canada, during three seasons. As a measure of concentrations of PBPAHs to which fishes are exposed and to gain information on the nature and extent of potential exposures of people or piscivorous wildlife, I used synchronous fluorescence spectroscopy to measure the concentrations of biotransformation products of 2 and 3-ringed, 4-ringed, and 5-ringed PAHs.

I have published this chapter as a first author in Environmental Geochemistry and Health (2015, DOI 10.1007/s10653-015-9744-6), under joint authorship with Brett Tendler (University of Saskatchewan), Allison Hill (University of Saskatchewan), Gary Codling (University of Saskatchewan), Erin Kelly (Government of the Northwest Territories), John P. Giesy (University of Saskatchewan), and Paul Jones (University of Saskatchewan). I played a leading role in the research design and sample collection, Mr Tendler, Miss Hill and Dr. Codling assisted with sample collection. I was solely responsible for the analysis of PBPAHs and I wrote and revised this manuscript with editorial comments from the other authors. Dr. Kelly was responsible for coordination with communities, the government of NWT and sponsors. Dr. Giesy provided part of the research funding and editorial feedback. Dr. Jones provided a substantial part of my student and research funding. Furthermore, he coordinated the field work, assisted with experimental design, data interpretation and provided editorial feedback.

CHAPTER 2

Products of Biotransformation of Polycyclic Aromatic Hydrocarbons in Fishes of the Athabasca/Slave River System, Canada

Abstract

Concentrations of products of biotransformation of polycyclic aromatic hydrocarbons (PBPAH) were measured in bile of five fishes of nutritional, cultural and ecological relevance from the Athabasca/Slave River system. Samples were collected in Alberta and the Northwest Territories, Canada, during three seasons. As a measure of concentrations of PBPAHs to which fishes are exposed and to gain information on the nature and extent of potential exposures of people or piscivorous wildlife, concentrations of biotransformation products of 2 and 3-ringed, 4-ringed, and 5-ringed PAHs were measured using synchronous fluorescence spectroscopy (SFS). Spatial and seasonal differences were observed with greater concentrations of PBPAHs in samples of bile of fish collected from Fort McKay as well as greater concentrations of PBPAHs in bile of fish collected during summer compared to those collected in other seasons. Overall, PBPAHs were greater in fishes of lower trophic levels and fishes more closely associated with sediments. In particular, goldeye (*Hiodon alosoides*), consistently contained greater concentrations of all the PBPAHs studied.

2.1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) in the Lower AR basin are released naturally from oil sands deposits and from production of synthetic oil during extraction and upgrading of oil sands (Akre et al., 2004; Gentes et al., 2006). Additional sources of PAHs include forest fires, urban run-off, and deposition from long-range, atmospheric transport from urban and industrial activities (Parajulee and Wania, 2014). Because of their carcinogenic and mutagenic properties (Deutsch-Wenzel et al., 1983) some of the many thousands of theoretically possible PAHs are classified as high-priority pollutants by regulatory agencies and their concentrations are monitored so that risks to humans and wildlife can be calculated.

Exploitation of the oil sands and associated development in Alberta is a cause of concern due to potential contamination of downstream reaches by the various constituents of bitumen (Dillon et al., 2011). These concerns have been centered on unintentional releases of oil sands process water (OSPW) from tailings ponds (Timoney and Lee, 2009; Gagné et al., 2011) as well as emissions from extraction and processing activities. Furthermore, it has been suggested by some that treated OSPW will eventually be released to surrounding surface waters (Kean, 2009), which could ultimately result in releases to the AR that might transport the materials downriver. Thus, it was deemed crucial to establish baseline values of relevant parameters before any discharge occurs. In addition, rapid expansion in the intensity and extent of oil sands activities can be expected to add to contaminant loadings to the environment. Thus, there is a need to assess the geographical extent of potential contaminant releases from activities in the oil sands because contaminants potentially released could be transported as far as the Great Slave Lake and even to the Mackenzie River basin and delta.

Monitoring of fish can be used as an integrative measure of concentrations of contaminants over time and be an indicator of possible risks from consumption of fish by humans or wildlife. The presence of PBPAHs in bile of fishes can be used as a surrogate to evaluate exposure to petroleum contaminants derived from bitumen. However, not all ambient bioavailable PAHs are accumulated and metabolized in fish to form soluble conjugates following epoxy and hydroxyl derivative formation. As such, PBPAHs can only be used as general indicator of concentrations of petroleum hydrocarbons and bitumen (Jung et al., 2011; Yang and Baumann, 2006). Concentrations of PBPAHs in bile of fishes have been used as a biomarker of exposure to hydrocarbons in both marine and freshwater systems and in laboratory studies (Vuorinen et al., 2006; Jung et al., 2011; Insausti et al., 2009; EscartÍn and Porte, 1999).

Measurement of PBPAHs in bile of fishes by use of fluorescence has several advantages over monitoring in other tissues of fishes or other environmental media. First, PBPAHs are accumulated in bile, allowing a time-integrated measurement of exposure. Since PBPAHs are eliminated relatively rapidly from the gall bladder, usually within less than a week, they provide an estimate of exposure to the respective parent PAHs over a relatively short duration prior to collection of the fish. The feeding strategy, such as benthic, supra-benthic, or pelagic and, trophic status, patterns of migration and habits of spawning of different fishes can affect exposure of fishes to PAHs (EscartÍn and Porte, 1999; EscartÍn and Porte, 1999). Rates of accumulation, biotransformation and excretion are important factors in determining the viability of using aquatic species for monitoring (Vuorinen et al., 2006; EscartÍn and Porte, 1999).

Synchronous fluorescence spectroscopy (SFS) has been adopted as an alternative to costlier and time-consuming techniques such as high performance liquid chromatography (HPLC) with fluorescence or mass spectrometric detection (EscartÍn and Porte, 1999; Leonard

and Hellou, 2001; McDonald et al., 1995). SFS is a rapid, sensitive and cost-effective technique useful for analyzing large numbers of samples without the need for extensive pre-treatment. This method has been used for a variety of purposes including characterizing natural organic matter in saline organic soils (Guo et al., 2013), detecting oil sands process-affected waters in the Alberta oil sands region (Kavanagh et al., 2009), monitoring of PAHs in biota (Jung et al., 2011; Vuorinen et al., 2006; Insausti et al., 2009). It has also been used to identify sources of petroleum contaminants (Pharr, McKenzie, and Hickman, 1992; Han et al., 2006).

The objective of this study was to assess potential exposure of fish to various classes of PBPAHs in the Athabasca/Slave river system by focusing on fishes that are, in addition to their ecological significance, of cultural, nutritional and economic significance to local consumers. Multiple species of fishes were collected to assess the potential for ecological and trophic variables to alter exposure estimates and to elucidate the environmental behavior of PBPAHs.

2.2 Materials and methods

2.2.1 Chemicals and Reagents

All glassware was hand washed and rinsed several times with deionized water, then solvent rinsed three times with hexane and dichloromethane before use. Deionized water was taken from a Milli-Q system. All solvents used were HPLC grade (Fisher Scientific, Canada). Quantification was calibrated by use of a seven-point calibration curve of pure standards (AccuStandard New Haven, USA) of anthracene (100-3000 ng/ml), chrysene (100-5000 ng/ml) and benzo(a)pyrene (0.1-50 ng/ml), representative of 2 and 3-ring, 4-ring, and 5-ring, PBPAHs respectively. The calibration standards were measured simultaneously with the samples.

2.2.2 Characterization of biotransformation products of PAH using SFS

Concentrations of PBPAHs in bile were determined by SFS. To avoid inner filter effects and other matrix effects, such as resonance energy transfer and collisional quenching, a 5 µL sample of bile was diluted 1000 fold with 50% methanol/H_2O (v/v) and centrifuged at 10,000 g for 15 min at 4 ^{0}C to remove particulates. The supernatant containing PBPAHs was analyzed in a quartz cuvette by use of a Thermo Scientific Lumina Fluorescence Spectrometer. The sum excitation/emission wavelength parameter rather than individual compounds were measured, scanning a range of 200 to 600 nm with a fixed wavelength differential of 42 nm (Aas and Klungsøyr, 1998). Each sample was quantified in triplicate. Measuring PBPAH levels using SFS is characteristic for each ring structure and this can be used for identification. Parent PAHs have similar fused benzene rings as their products of biotransformation, and are detected at specific excitation/emission wavelengths (Insausti et al., 2009). Differences in fluorometric properties of the PBPAH ring structures present in a supernatant can be optimized to acquire semi-quantitative

measures of the PBPAH ring present (Vuorinen et al., 2006). Fluorescence, in samples and standards, was detected at 290/335nm for 2 and 3-ring 341/383 for 4-ring and 380/430nm for 5-ring PBPAHs. Results are expressed in "equivalents" of these reference compounds. Biliverdin was quantified at 380 nm. To avoid additional uncertainty in the data, concentrations of PBPAHs are presented un-normalized to either biliverdin or protein. This is acceptable for discriminating exposed from non-exposed organisms (Aas and Klungsøyr, 1998; Vuorinen et al., 2006).

2.2.3 Quality control and Quality assurance

All analytical data were subject to strict quality control. Method blanks (A blank with 50% methanol was subtracted from the standards and samples.), and spiked blanks (standards spiked into solvent) were used to determine any background contamination, which showed no detectable PAHs. Some samples were prepared and analyzed in duplicate. The instruments were calibrated frequently with calibration standards. The method detection limit was defined as three times the blank measurement (Beyer et al., 2010).

2.2.4 Statistical analyses

Fish size index and equivalent concentrations of PBPAHs are presented as mean $\pm$ standard deviation (SD). Frequency distributions (histogram) and boxplots were used to check for normality. Parametric one-way analysis of variance (ANOVA) was used to test for differences among locations, season, species, and sexes. Significance was set at $p < 0.05$. Pearson's correlation analysis was used to test the relationships between mass, length, LSI, and concentrations of PBPAHs in bile. Data were displayed by use of Box-Whisker plots (McGill et al., 1978).

2.3 Results

In total, 565 fish were collected from five locations over three seasons (Table 2.1). Natural history characteristics of the five species collected, trophic level, primary diet type, habitat and spawning season are provided (Table 2.1). Goldeye, walleye and whitefish were the predominant fishes collected at all five locations, during all seasons. Northern pike was also well represented among locations. Burbot was collected primarily from the SR. Of the PBPAHs monitored, 2 and 3-ring PBPAHs were detected in all fishes analyzed, while 4-ring PBPAHs were detected in most samples of bile analyzed, while 5-ring PBPAHs were widespread in whitefish, walleye and goldeye predominantly in locations near oil sands operations.

Table 2.1: Latin names, trophic positions and numbers of individuals of each target species collected for the purpose of this study.

	Jackfish	Goldeye	Whitefish	Walleye	Burbot
Scientific Name	*Esox lucius*	*Hiodon alosoides*	*Coregonus clupeaformis*	*Sander vitreus*	*Lota lota*
Trophic Level	4.4 ±0.7	3.0±0.4	3.1±0.4	4.5±0.5	4.0±0.7
Diet Type	Benthic/suprabenthic	Pelagic	Pelagic/benthic	Benthic/suprabenthic	Benthc/suprabenthic
Basin Wide	No	Yes	Yes	Yes	Med
Migratory	No: usually solitary	Yes: potamodrous	Yes: anadromous	Yes; potamodromous	Med: potamodromous
Spawns	Spring	Spring	Fall	Fall	Fall/Winter
Human Diet	Yes	Yes	Yes	Yes	Yes
Habitat	Shallow (usually 1-5 m), occurs in clear vegetated lakes	Turbid slow moving waters, also found in muddy shallow areas of lakes	Primarily a lake dweller. Neritopelagic	Prefers large, shallow lakes with turbidity	Benthic. Secluded under boulders, submerged macrophytes, or in the organic flocculent on substrate.
Fort McMurray	23	21	14	23	6
Fort McKay	24	30	22	30	2

Fort Chipewyan	30	30	30	25	5
Fort Smith	30	30	23	30	9
Fort Resolution	30	22	30	20	26

2.3.1 Total concentrations of PBPAH and indices of fishes

Biological parameters including the number of individuals collected on each sampling site, total mass and length are provided in Appendix A, respectively. In summer, burbot collected at Fort Resolution had a mean length of 62 cm and mass of 1591 g. But these values decreased upstream, during the same sampling season. For instance, at Fort McMurray mean length of burbot was 41 cm and mass was 420 g. The mean mass of goldeye collected from Fort Resolution was 646 g compared to those at Fort McKay which had a mean mass of 685 g and mean lengths of 38.1 cm and 37.94 cm, respectively. Across all sampling sites Jackfish sampled during the summer months had similar size and mass averaging 64 cm and 2032 g, respectively. Walleye showed a uniform length/mass distribution in the sampling locations. The size of whitefish varied from 1296 g to 1018 g and length, 44 cm to 39 cm. Goldeye were slightly greater upstream of Fort Resolution (35.8 cm, 546 g) to Fort McMurray (39.37 cm, 700 g). Biological parameters for burbot increased downstream during the spring sampling period. In general, no consistent size or mass differences were observed among locations, a result that suggests that sampled fishes were of similar age/year.

2.3.2 Differences in Concentrations of BPPAHs among Locations

Concentrations of classes of PBPAHs in the 5 species at the different sampling locations are reported (Table 2.2). Mean concentrations of the sum of PBPAHs, averaged across the five species, were greatest at Fort McKay, followed by Fort McMurray and Fort Chipewyan. Total concentrations were least at Fort Resolution and Fort Smith. Significant differences were observed among sites for 2 and 3-ring PBPAHs ($F_{5.88}$=3.15; p = 0.0004) and for 5-ring PBPAHs

($F_{6.42}$ = 3.15, p = 0.0002). No significant differences were observed among locations for 4-ring PBPAHs ($F_{1.71}$ = 3.15, p = 0.15).

Table 2.2: Mean concentrations (± SD) of products of biotransformation of polycyclic aromatic hydrocarbons (PBPAHs) (ng/ml) measured by synchronous fluorescence spectroscopy (SFS) in fishes collected at Fort Resolution, Fort Smith, Fort Chipewyan, Fort McKay, and Fort McMurray in a) summer 2011, b) fall of 2011 and c) spring of 2012.

Table 2.2 a) Summer 2011

Species	Site	2-3 ring	4-ring	5-ring	Total
Burbot	F.Resolution (10)	1509 ± 742	639 ± 281	4.7 ± 2.5	2151 ± 940
	F.Smith (5)	2341 ± 1872	1012 ± 295	2.4 ± 1.0	3355 ± 2087
	F.Chipewyan(2)	5567 ± 277	1947 ± 1747	0.0 ± 0.0	7513 ± 2024
	F.McKay	n.a	n.a	n.a	n.a
	F.McMurray (3)	5655 ± 2951	2183 ± 526	0.0 ± 0.0	7839 ± 2592
Goldeye	F.Resolution (2)	3206 ± 258	2022 ± 522	1.4 ± 0.7	5230 ± 781
	F.Smith (10)	2390 ± 1026	2855 ± 1268	1.6 ± 0.7	5248 ± 1813
	F.Chipewyan (10)	2673 ± 2110	3036 ± 1177	2.0 ± 1.8	5711 ± 3026
	F.McKay (10)	3144 ± 1113	1464 ± 1235	1.2 ± 0.5	4608 ± 1963
	F.McMurray (10)	3474 ± 841	1999 ± 755	22 ± 8.7	5494 ± 1184
Jackfish	F.Resolution (10)	1144 ± 527	417 ± 144	1.1 ± 0.4	1561 ± 583
	F.Smith (10)	940 ± 387	467 ± 192	4.7 ± 2.1	1356 ± 584
	F.Chipewyan (10)	2582 ± 895	1286 ± 423	5.3 ± 3.6	3870 ± 1266
	F.McKay (10)	2670 ± 625	1200 ± 473	8.3 ± 5.5	3876 ± 1052
	F.McMurray (10)	2660 ± 2019	1170 ± 840	11.0 ± 11.3	3839 ± 2741
Walleye	F.Resolution	n.a	n.a	n.a	n.a
	F.Smith (10)	3099 ± 575	1138 ± 1077	5.6 ± 6.4	4240 ± 883
	F.Chipewyan (10)	3463 ± 1196	976 ± 410	5.9 ± 1.8	4402 ± 1276
	F.McKay (10)	3446 ± 1454	1239 ± 655	19.5 ± 7.1	4699 ± 2001

	F.McMurray (10)	3949 ± 1130	1546 ± 863	17.8 ± 9.9	5510 ± 1824
Whitefish	F.Resolution (10)	2657 ± 623	652 ± 206	1.1 ± 0.6	3310 ± 648
	F.Smith (8)	2036 ± 1058	1033 ± 313	3.7 ± 5.5	3073 ± 1228
	F.Chipewyan (10)	4660 ± 1472	2771 ± 1336	2.0 ± 0.6	7433 ± 2583
	F.McKay (10)	5165 ± 1831	2893 ± 617	24.5 ± 7.0	8082 ± 2425
	F.McMurray	n.a	n.a	n.a	n.a

Table 2.2 b) Fall 2011

Species	Site	2-3 ring	4-ring	5-ring	Total
Burbot	F.Resolution (10)	1311 ± 839	438 ± 210	1.6 ± 1.4	1750 ± 979
	F.Smith (3)	1161 ± 581	607 ± 122	1.4 ± 0.4	1768 ± 562
	F.Chipewyan (3)	1381 ± 551	748 ± 136	1.4 ± 0.0	2130 ± 606
	F.McKay (3)	2866 ± 1909	906 ± 386	13.2 ± 0.0	3779 ± 2305
	F.McMurray	n.a	n.a	n.a	n.a
Goldeye	F.Resolution (10)	1625 ± 972	879 ± 520	3.4 ± 3.3	2343 ± 1331
	F.Smith (10)	1190 ± 808	703 ± 345	7.8 ± 4.6	1900 ± 850
	F.Chipewyan (10)	1430 ± 933	879 ± 933	6.1 ± 9.0	2314 ± 1607
	F.McKay (10)	2971 ± 876	1010 ± 907	0.8 ± 0.3	3981 ± 1375
	F.McMurray (1)	2600 ± 0.0	770 ± 0.0	18.1 ± 0.0	3388 ± 0.0
Jackfish	F.Resolution (10)	989 ± 222	489 ± 143	0.7 ± 0.3	1479 ± 309
	F.Smith (10)	1008 ± 578	463 ± 203	1.8 ± 2.1	1420 ± 813
	F.Chipewyan (10)	1482 ± 831	603 ± 238	4.1 ± 4.4	2086 ± 1036
	F.McKay (10)	2651 ± 1004	718 ± 341	8.7 ± 1.3	3371 ± 1092
	F.McMurray (10)	2428 ± 1332	535 ± 481	n.a	2962 ± 1217
Walleye	F.Resolution (10)	2697 ± 1638	685 ± 372	2.7 ± 4.0	3394 ± 2001
	F.Smith (10)	2267 ± 1293	791 ± 290	4.4 ± 6.9	3063 ± 1479
	F.Chipewyan (5)	3104 ± 507	1315 ± 766	16.2 ± 14.3	4431 ± 1074
	F.McKay (10)	3192 ± 1154	852 ± 579	10.9 ± 4.4	4052 ± 1584
	F.McMurray (3)	3176 ± 449	879 ± 183	6.5 ± 4.8	4059 ± 626

Whitefish	F.Resolution (10)	829 ± 815	524 ± 352	1.1 ± 0.4	1237 ± 1114
	F.Smith (10)	1683 ± 991	849 ± 447	4.1 ± 4.7	2354 ± 1382
	F.Chipewyan (10)	2640 ± 489	914 ± 389	0.9 ± 0.3	3554 ± 699
	F.McKay (10)	2402 ± 748	1256 ± 518	13.2 ± 7.1	3545 ± 834
	F.McMurray (10)	1587 ± 708	649 ± 454	0.9 ± 0.4	2236 ± 1088

Table 2.2 c) Spring 2012

Species	Site	2-3 ring	4-ring	5-ring	Total
Burbot	F.Resolution (6)	893 ± 0.0	435 ± 0.0	1.0 ± 0.0	1329 ± 0.0
	F.Smith (1)	385 ± 0.0	327 ± 0.0	2.1 ± 0.0	714 ± 0.0
	F.Chipewyan	n.a	n.a	n.a	n.a
	F.McKay	n.a	n.a	n.a	n.a
	F.McMurray (3)	2338 ± 210	947 ± 714	10.1 ± 0.0	3288 ± 793
Goldeye	F.Resolution (10)	919 ± 567	616 ± 738	4.2 ± 4.2	1538 ± 1103
	F.Smith (10)	1297 ± 654	874 ± 339	6.3 ± 5.1	2176 ± 789
	F.Chipewyan (10)	2289 ± 786	871 ± 579	5.8 ± 6.8	3164 ± 910
	F.McKay (10)	2678 ± 895	1442 ± 1006	14.6 ± 9.0	4135 ± 900
	F.McMurray (10)	1687 ± 993	1211 ± 374	15.6 ± 6.9	2913 ± 1298
Jackfish	F.Resolution (10)	659 ± 381	336 ± 155	0.9 ± 0.5	921 ± 571
	F.Smith (10)	1055 ± 587	432 ± 78	0.0 ± 0.0	1487 ± 622
	F.Chipewyan (10)	1330 ± 534	432 ± 205	0.0 ± 0.0	1762 ± 664
	F.McKay (5)	1306 ± 760	530 ± 229	4.3 ± 1.4	1838 ± 767
	F.McMurray (10)	1546 ± 632	591 ± 245	5.4 ± 1.7	2142 ± 839
Walleye	F.Resolution (10)	2021 ± 544	331 ± 69.8	0.0 ± 0.0	2352 ± 553
	F.Smith (10)	1978 ± 571	554 ± 258	0.9 ± 0.1	2532 ± 427
	F.Chipewyan (10)	2211 ± 630	515 ± 229	5.8 ± 4.2	2731 ± 777
	F.McKay (5)	2455 ± 591	512 ± 317	4.9 ± 5.7	2972 ± 722

	F.McMurray (10)	2096 ± 582	566 ± 457	5.9 ± 2.9	2667 ± 782
Whitefish	F.Resolution (10)	1683 ± 504	925 ± 1033	1.4 ± 0.9	2608 ± 1350
	F.Smith (5)	1344 ± 800	988 ± 460	1.0 ± 0.2	2333 ± 1220
	F.Chipewyan (10)	2773 ± 1204	318 ± 128	0.4 ± 0.0	3091 ± 1324
	F.McKay (2)	3183 ± 2039	1078 ± 473	3.8 ± 3.7	4265 ± 1570
	F.McMurray (4)	2313 ± 354	826 ± 342	5.0 ± 5.1	3144 ± 639

2.3.3 Seasonal Variation

When concentrations of PBPAHs were compared among species stratified by location and season (Table 2.3), all the metabolites exhibited some variation among seasons. Greater concentrations were observed in summer than in spring and fall. When species were compared within seasons; 2 and 3-ring PBPAHs ($F_{12.48} = 4.15$, $p = 0.00003$) summer > fall = spring; for 4-ring PBPAHs ($F_{19.36} = 4.15$, $p = 0.0000002$) summer > fall = spring; for 5-ring PBPAHs ($F_{0.88} = 4.15$, $p = 0.42$) summer = fall = spring. Concentrations were greatest in fishes from near Fort MacKay, and Fort McMurray with lesser concentrations at downstream locations. This profile was consistent among seasons. However, absolute concentrations of PBPAHs were greater in summer than spring and fall. This variation among seasons was consistent for all PBPAHs but differences among seasons were greatest for 4-ring PBPAHs.

2.3.4 Differences among Species

Differences in concentrations of PBPAHs among species were investigated by pooling individuals from all sampling dates. No significant difference was observed among species ($p <$

0.05). Walleye and whitefish exhibited the greatest concentrations of 2 and 3-ring PBPAHs in bile. For the 4-ring PBPAHs, goldeye and whitefish contained greater concentrations in their bile, while walleye and goldeye contained the greatest concentrations of 5-ring PBPAHs in bile.

Table 2.3: Concentrations (Mean $\pm$ SD) of products of biotransformation of polycyclic aromatic hydrocarbons (PBPAHs) in five fishes from the Athabasca and Slave Rivers during three seasons. Statistical differences between pairs of seasons ($p < 0.05$) for 2-3-ring, 4-ring and the total concentration of PBPAHs are indicated by different letters. There was no statistical difference between pairs of seasons ($p < 0.05$) for 5-ring PBPAHs. All values are in ng/ml wet mass (wm).

	Season	2-3-ring	4-ring	5-ring	Total
Burbot	Summer (20)	3768 ± 2155	1445 ± 738	1.7 ± 2.2	5215 ± 2888
	Fall (18)	1680 ± 796	675 ± 200	4.4 ± 5.8	2357 ± 964
	Spring (10)	639 ± 359	381 ± 76	1.5 ± 0.8	468 ± 348
Goldeye	Summer (42)	2977 ± 437	2275 ± 654	5.7 ± 9.2	5258 ± 414
	Fall (41)	1963 ± 778	848 ± 118	7.2 ± 6.6	2600 ± 1067
	Spring (50)	1774 ± 716	1003 ± 324	9.3 ± 5.4	2785 ± 989
Jackfish	Summer (40)	1999 ± 878	908 ± 428	6.1 ± 3.7	2900 ± 1318
	Fall (42)	1711 ± 785	561 ± 102	3.1 ± 3.5	2193 ± 926
	Spring (45)	1179 ± 339	464 ± 99	2.1 ± 2.5	1630 ± 460
Walleye	Summer (40)	3489 ± 877	908 ± 428	6.1 ± 3.7	2900 ± 1318
	Fall (38)	2887 ± 401	906 ± 239	8.2 ± 5.5	3277 ± 1257
	Spring (50)	2152 ± 191	496 ± 95	3.5 ± 2.8	2651 ± 231

Whitefish	Summer (38)	3629 ± 1517	1838 ± 1160	7.8 ± 11.2	5475 ± 2652
	Fall (50)	1828 ± 719	838 ± 281	4.0 ± 5.3	2597 ± 1018
	Spring (31)	2259 ± 757	827 ± 299	2.3 ± 2.0	3088 ± 739

2.3.5 Differences between Sexes

During all samplings, males and females were caught. Beside the significant difference observed between male and female burbot within fall, there was no statistically significant difference in concentrations of the classes of PBPAHs, between male and female among the five fishes (Table 2.4).

2.3.6 Concentrations of PBPAHs in Bile of Jackfish

Northern pike (*Esox luscious*; jackfish) rarely travel significant distances, this territorial behavior makes them a more suitable indicator species for localized contamination (Fig. 2.1). Mean concentrations of PBPAHs in jackfish of the three seasons were greatest at Fort McKay, followed by Fort McMurray (Fig. 2.1). The range of data was greater in individuals from Fort McMurray, Fort McKay and Fort Chipewyan. During summer; concentrations of PBPAHs in Fort McMurray and Fort McKay were significantly greater than those of Fort Smith and Fort Resolution ($F_{13.6} = 3.04$, $p << 0.0001$).

Table 2.4: Mean values of biotransformation products of PAHs, standard deviations (SD) and P-values (0.05) comparing Sexs of fishes within seasons.

		Summer	Fall	Spring	Total
Burbot	Female	3662 ± 3126	1620 ± 902	1021 ± 435	2101 ± 1385
	Male	4851 ± 3014	2811 ± 1443	4200 ± 0	3954 ± 1042
	P-Value	0.52	0.04	0.11	0.14
Goldeye	Female	5549 ± 2163	2637 ± 1385	2632 ± 1294	3606 ± 1683
	Male	4956 ± 1710	3695 ± 1671	3310 ± 1159	3987 ± 861
	P-Value	0.46	0.38	0.33	0.74
Jackfish	Female	2498 ± 1664	2444 ± 1207	1749 ± 726	2230 ± 418
	Male	3029 ± 1695	2394 ± 1241	1705 ± 1024	2376 ± 662
	P-Value	0.31	0.91	0.91	0.76
Walleye	Female	4735 ± 1258	4307 ± 1583	2751 ± 684	3931 ± 1043
	Male	4831 ± 1767	3438 ± 1396	2548 ± 680	3606 ± 1151
	P-Value	0.87	0.08	0.36	0.74
Whitefish	Female	5484 ± 2877	3034 ± 1129	2935 ± 1292	3818 ± 1443
	Male	5602 ± 3142	2363 ± 1315	2958 ± 1280	3641 ± 1724
	P-Value	0.91	0.07	0.96	0.89

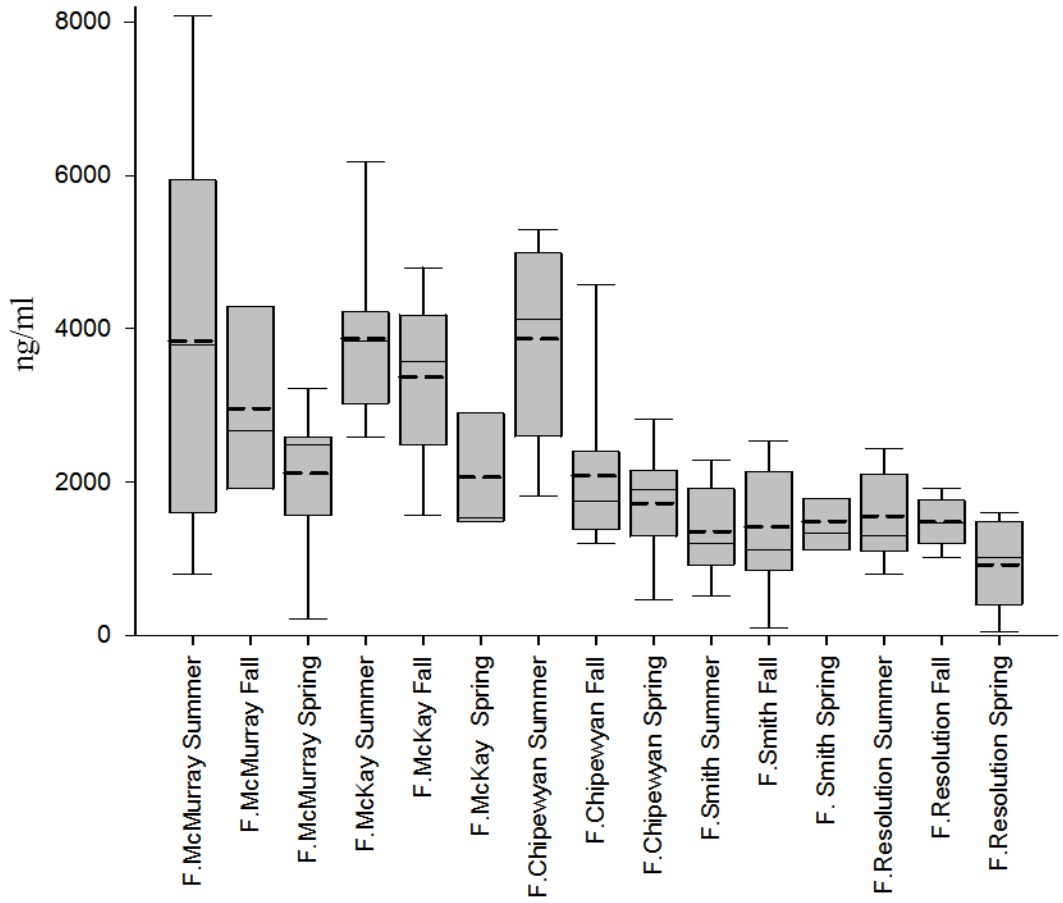


Figure 2.1: Box plots showing the spread of concentrations (ng/ml, wet mass) of biotransformation products of PAH in bile of jackfish from the five sampling areas, during three seasons. Confidence interval is 95%. Thick line is the median, dotted line is the mean. The width of the box shows the interquartile range. The top 50% of the concentration are represented by everything above the median. The top 25% concentrations are shown by the top whisker.

2.4 Discussion

Variation within the sampled populations might be related to the feeding status of individuals and locations where they were caught. Preferences for habitat vary among species from benthic to pelagic environments (Table 2.1). Jackfish tend to be sedentary and territorial, feeding in a relatively small area, which makes this species useful for spatial comparison along the basin. However, the relatively rapid elimination of PBPAHs also means that observed concentrations are most indicative of recent feeding even in migratory species.

The type of food consumed might influence concentrations of PBPAHs in bile. The fishes studied were assigned to trophic levels 2 to 4 (Walker et al., 2012). Lake whitefish is a first order carnivore; but they are occasional piscivorous, consuming small fishes (Scott, Crossman, 1979; Nelson, Paetz, 1992). Burbot, walleye and jackfish are piscivores (Braune et al., 1999). It is common for piscivorous fishes to have greater concentrations of some contaminants because they obtain pollutants via both food and partitioning from water (Borga et al., 2004). Furthermore, piscivores generally live longer than their prey species, which increases their duration of exposure (Larsson et al., 1992). However, because they have relatively short half-lives due to biotransformation, PAHs do not exhibit biomagnification, but rather exhibit biodiminution between trophic levels (Walker et al., 2012). In this study, whitefish and goldeye demonstrated greater concentrations of PBPAHs than did higher trophic level species, which is consistent with expectations considering the trophic levels of the species studied. Both lake whitefish and goldeye are bottom and pelagic feeders respectively, consuming a wide range of invertebrates, primarily clams, snails, gastropods, and chironomid larvae. Some of these invertebrates bioconcentrate and/or bioaccumulate PAHs because they have little ability to transform them (Walker et al., 2012). Thus, fishes that feed on invertebrates are more likely to be

exposed to greater concentrations of PAHs. Conversely, burbot and jackfish are mainly piscivores; and their prey, which are fishes that can biotransform and eliminate PAHs relatively rapidly, reduces their potential for accumulations of PBPAHs in the bile (Megenau et al., 1998).

The concentrations of PBPAHs observed in walleye were unexpected based on trophic status alone. But other factors could be responsible for the greater concentrations observed in this species. Fish ecology, preferred habitat, and diet usually influence exposures of fishes to PAHs (Kidd et al., 1998; Tang et al., 2013; Borga et al., 2004). Species with preferences for benthic habitats are more likely to be exposed to PAHs in a polluted environment. Because PAHs are lipophilic, in aquatic environments, they tend to sorb and accumulate in available organic phases such as the tissues of aquatic organisms (Timoney and Lee, 2011; Wayland et al., 2008) and the organic phases of sediments, soils and biota (McCarthy et al., 1997). Walleye prefer mesotrophic environments (Kerr et al., 1997), and live in aquatic vegetation in turbid environments for foraging and protection from sunlight (Rieger and Summerfelt, 1997). This provides close contact with sediments. Walleye are thus exposed to organic pollutants such as PAHs accumulated in sediments and their food items. Lake whitefish are bottom feeders (Johnson et al., 2009), burbot live near the bottom of lakes (Polacek et al., 2006), and northern pike prefer habitat that is shallow and vegetation-rich (Margenau et al., 1998) which results in greater exposure to bioaccumulative contaminants in more polluted areas.

It has been suggested that in the oil sands region contaminants accumulated in ice and snow from aerial deposition of dust and other emissions could result in a spring pulse of contaminants into rivers and lakes (Kelly et al., 2009). But our study with fishes in the area did not show a similar trend with PBPAHs measured in the bile of the sampled fishes. Differences among seasons observed for individual PBPAHs and their sum can be associated with

environmental variables such as temperature, reproductive cycle, and receiving water dilution factors (Coat et al., 2011; Simonin et al., 2008; Borga et al., 2004). Like other organic contaminants, 23^0C is the optimal temperature for uptake, biotransformation, and elimination of PAHs in some fish species (Jimenez et al., 1987). During summer, the temperature ranged from 19^0C to 24^0C (RAMP, 2012). For some of these species, optimal gonad development occurs in summer and they spawn in fall (Table 2.1) (Scott and Crossman, 1979). Concentrations of PAHs, like other lipophilic compounds, are greater in these fishes during gonad development (Evans et al., 2005). It is also possible that concentrations of PAHs in the environment can be diluted by melting ice and snow during spring (Kelly et al., 2009). Although water currents can re-suspend sediments and associated PAHs and increase bioavailability during spring, the increase is diluted by the runoff from snowmelt and rainfall.

In this study, maximum concentrations of PBPAHs were observed in the vicinity of Fort McKay. Processing of oil sands, which takes place near Fort McMurray could be a significant source of PAHs at downstream locations. Facilities where oil sands are processed are located just upstream from Fort McKay, which could explain why Fort McKay and Fort Chipewyan had greater concentrations of the sum of PBPAHs that were studied. Greatest concentrations were observed in the vicinity of Fort McMurray, which is upstream from the processing facilities. This observation might be a result of aerial deposition from processing of oil sands (Kelly et al., 2009) or to runoff from streets and in wastewater effluents, both of which are known to be sources of PAHs in surface waters. Also, the McMurray Formation underlies this site upstream and some areas of the AR in that region have incised into this formation (Conly, Crosley, and Headley, 2002), likely contributing natural increases in concentrations of petroleum derived PAHs. However, observation of greater concentrations of PBPAHs in bile of fishes of the Lower

Athabasca basin does not necessarily indicate a cause-effect relationship to tar sands presence and operations. The source of PBPAHs in the study area might also be pyrogenic (from incomplete combustion of organic matter and fossil fuel) (Abrajano et al., 2003; Nkpaa et al., 2013). Forest fires are also frequent in the study area (Wayland et al. 2008; Conly and Headley, 2002) and could be an additional source.

Though no clear distinction exists, in general, mixtures of PAHs from petrogenic sources contain greater proportions of LMW-PAHs (e.g., naphthalene and acenaphthenes) (Nkpaa et al., 2013), while pyrogenic sources have greater proportions of HMW-PAHs (e.g., benzo[a]pyrene), (Lin et al., 1996; Soclo et al., 2000). Fish exposed to petrogenic contaminants will likely have relatively greater proportions of 2- and 3-ring PBPAHs in bile (Ruddock et al., 2002). 2- and 3-ring PBPAHs predominated in bile of all five species among all three seasons. 4-ring PBPAHs were also major constituents in bile of fishes from the Athabasca/Slave River system while concentrations of 5-ring PBPAHs were least. The concentration and distribution of PBPAHs observed in this study is indicative of parent PAHs from petrogenic sources within the Athabasca basin (Headley et al., 2002). It is however unclear if the petrogenic source came from development or from seeps of natural bitumen, as both would likely have similar relative proportions of PBPAHs.

The presence of 5-ring PBPAHs in bile of fishes is especially important because of the carcinogenic and mutagenic properties of some of the compounds in this class. Compared to other PBPAHs, 5-ring PBPAHs are not readily bioavailable because of their relatively small solubility in water and thus concentrations of these PAHs and their products of biotransformation in environmental samples are usually less than PBPAHs with 2,3, or 4-rings (Ruddock et al., 2002; Insausti et al., 2009). But, 5-ring PAHs can persist in aquatic environments and

bioaccumulate in fish because they are less readily biotransformed by indigenous microorganism. For this reason, they are regarded as indicators of exposure of aquatic organisms to PAHs.

All of the fishes studied, with the exception of jackfish, are migratory to some extent (Table 2.1), nevertheless, the observed results were indicative of the ambient environment where the fishes were caught. Long-range movement might result in exposure to contaminants, directly through bioconcentration of dissolved pollutants or to contaminants accumulated in the food chain, at different locations along the migratory route. But migration will likely be of lesser significance in evaluating sources of PBPAH, due to the relatively great rates of elimination and short half-lives of these compounds (Jimenez et al., 1987). This presumably indicates uptake of PAHs mainly through absorption from the diet, attributable to contamination of their prey. Concentrations of PBPAHs in bile of fishes is a function of exposure during the previous 2-3 days (Ariese et al., 1993). Moreover, jackfish are a relatively sedentary species that provide a good indicator for spatial comparison (Fig. 2.1). The results from jackfish demonstrate greater concentrations of PAHs in the aquatic environment in the vicinity of Fort McMurray, and Fort McKay compared to Fort Smith and Fort Resolution.

2.5 Conclusions

The results reported here provide baseline data on concentrations of PBPAH, measured by SFS in five species of large-bodied fishes consumed by humans in communities in the Lower Athabasca/Slave River basin. Spatial and temporal trends in profiles of relative concentrations of PBPAH, coincided with fishes in locations proximate to oil sands operations. Trophic level and habitat preference of selected fishes were also important factors in accounting for the

concentrations of PBPAHs in bile of fishes. These results will be valuable for establishing the status of trends and spatial distribution of PAHs during monitoring of the lower Athabasca basin and most importantly, as a valuable reference point before any potential permitted discharges of wastewaters from processing of oil sands to the AR. Further research would be required to determine risks posed by current concentrations of PBPAHs to humans. Since humans mainly eat muscle tissues (not bile) it would be necessary to establish the relationships between and among concentrations of PBPAHs in bile with those in edible portions of fishes and to be able to extrapolate these for use in assessment of risks to health of humans. In the future, the protocol we utilized in this paper will provide a relatively rapid method for monitoring PAHs and products of their biotransformation the Athabasca area.

Preface to Chapter 3: Potential Health Risks Posed by Polycyclic Aromatic Hydrocarbons in Muscle Tissues of Fishes from the Athabasca and Slave Rivers, Canada

Since humans mainly eat muscle tissues (not bile) it was necessary to establish the concentration of PAHs in edible portions of fishes and be able to extrapolate these for use in assessment of risks to health of humans. My second objective was to determine the levels of parent PAHs in the muscle of fish, and extrapolate the data to estimate potential risk to human consumers, and to identify which species and geographic regions, if any, pose the greatest risk to humans**.** Concentrations of 16 USEPA priority PAHs were measured in tissues of fishes collected from three locations on the AR in Alberta and two downstream locations on the SR in the Northwest Territories, Canada.

I have published this chapter as a first author in Environmental Geochemistry and Health (2016, DOI: 10.1007/s10653-016-9815-3), under joint authorship with Brett Tendler (University of Saskatchewan), Gary Codling (University of Saskatchewan), Erin Kelly (Government of the Northwest Territories), John P. Giesy (University of Saskatchewan), and Paul Jones (University of Saskatchewan. I played a leading role in the research design and sample collection, Mr. Tendler and Dr. Codling assisted with sample collection. I was solely responsible for the analysis of parent PAHs and I wrote and revised this manuscript with editorial comments from the other authors. Dr. Kelly was responsible for coordination with communities, the government of NWT and sponsors. Dr. Giesy provided part of the research funding and editorial feedback. Dr. Jones provided a substantial part of my student and research funding. Furthermore, he coordinated the field work, assisted with experimental design, data interpretation and provided editorial feedback.

CHAPTER 3

Potential Health Risks Posed by Polycyclic Aromatic Hydrocarbons in Muscle Tissues of Fishes from the Athabasca and Slave Rivers, Canada.

Abstract

Polycyclic aromatic hydrocarbons (PAHs) are released to the environment from oil sands operations and from natural sources in Alberta, Canada. Concentrations of 16 USEPA priority PAHs were measured in tissues of fishes collected from three locations on the Athabasca River in Alberta and two downstream locations on the Slave River in the Northwest Territories, Canada. A total of 425 individual fish were collected including 89 goldeye (*Hiodon alosoides*), 93 whitefish (*Coregonus clupeaformis*), 104 northern pike/jackfish (*Esox lucius*), 96 walleye (*Sander vitreus*), and 43 burbot/loche mariah/mariah (*Lota lota*). Fish were sampled during the summer and fall of 2011, and spring of 2012. Dorsal muscle of fishes from upstream reaches of the Athabasca River close to oil sands extraction and upgrading activities, contained greater concentrations of individual PAHs than concentrations in muscle of fishes from further downstream in the Slave River. Concentrations of the sum of US EPA indicator PAHs (∑PAHs) in fishes collected in the vicinity of Fort McKay, closest to oil sands activities, varied among seasons with average concentrations ranging from 11 (burbot, summer) to 120 ng/g, wm (burbot, spring) with a mean of 48 ng/g, wm. Concentrations of ∑PAHs in fishes collected in the vicinity of Fort Resolution, the location most distant from oil sands activities, also varied among species and seasons, with average concentrations ranging from 4.3 (whitefish, summer) to 33 ng/g, wm (goldeye, summer) with a mean of 13 ng/g, wm. Significant differences in concentrations of ∑PAHs in muscle were observed within goldeye, jackfish, walleye and whitefish among sites. Health Risks posed by PAHs to humans were assessed probabilistically using a B[a]P equivalents approach ($B[a]P_{eq}$). The average lifetime risk of additional cancers for humans who consumed fish was less than 10^{-6}.

3.1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a class of organic compounds composed of two or more fused aromatic rings, which are released to the environment by both human activities, and by natural events. PAHs are contaminants formed during the incomplete combustion of organic material and are also especially abundant in petroleum deposits and can also be released during operations involving the extraction, transport or processing of petroleum (Pampanin and Sydnes 2013).

The Alberta oil sands in Canada contain the second largest proven petroleum hydrocarbon reserves in the world, totaling an estimated 173.2 billion barrels of recoverable oil (Government of Alberta 2013). A large proportion of these reserves are found in deposits of bitumen, which cover approximately 60,000 km^2 (http://osip.alberta.ca/map/). Global demand for oil was 84.7 million barrels per day in 2008 and is expected to reach 105 million barrels per day by 2030 (International Energy Outlook 2013). Conventional production of crude oil is unable to meet this rising global demand for the readily available energy in petroleum hydrocarbons. Nonconventional oil sources including deposits of oil sands in Canada are required for a safe and secure energy future for North America and for the rest of the world. The global demand for Canadian oil has resulted in economic benefits for the country (Timilsina et al. 2005). The extensive development of the oil sands has also contributed to increased deposition of PAHs to the AR and its tributaries (Cho et al., 2014; Parajulee and Wania 2014). Dissolved polycyclic aromatic compound (PAC) concentrations up to 4.8 µg/L has been reported in melted snow collected from AR and its tributaries (Kelly et al. 2009). Concentrations of PAHs in sediments from Lake Athabasca and Lake Richardson, in the Peace/Athabasca delta, ranged from 1259-1867 µg/kg wet mass (WM) (Evans et al. 2002). Total concentrations of PAHs in sediments of

the AR Delta have reportedly increased between 1999 and 2009 at a rate of 0.05 mg/kg/yr (Timoney and Lee 2011).

Parts of the deposits of bitumen are in close proximity to the AR and its tributaries, thereby contributing hydrocarbons to the river. Some residents of downstream communities, especially in Fort Chipewyan, Alberta, on the shore of Lake Athabasca, have expressed concern that oil sands activities are contaminating country foods such as fish and game by contributing greater than natural levels of PAHs to the ambient environment (Timoney and Lee 2009; Chen 2009). Since fish is a major cultural and economic resource, the presence of PAHs in fishes of the Athabasca/Slave River system raises issues about potential risks to the health of humans in Aboriginal communities in the area (Usydus et al. 2009). Generally, dietary exposure to elevated concentrations of PAHs has been associated with increased risk of cancer in humans (Stacewicz-Sapuntzakis et al. 2008; Yoon et al. 2007). Some PAHs, such as benzo[a]pyrene, chrysene, indeno(1,2,3-c,d)pyrene and benzo(b)fluoranthene are known carcinogens. They also produce mutagenic, and genotoxic effects in experimental animals (Deutsch-Wenzel et al. 1983; Thyssen et al. 1981). Potential health risks of fish consumption need to be balanced with the proven benefits of the consumption of essential omega-3, unsaturated fatty acids and minerals in fish which have many health benefits including the reduction of coronary heart disease and lessening of hypertension (Sidhu 2003, Berry 1997).

Although there are regulatory and monitoring activities in the Athabasca basin, studies of PAHs in the region are few, making little data available for assessment of baseline concentrations of contaminants or effects on populations of fishes or the people who consume them (RAMP 2009 & 2012). Stakeholders have, in past years, expressed the need for the establishment of a comprehensive and transparent monitoring program in the AR (Dillon et al.

2011; Giesy et al. 2010; Weinhold 2011). Good reasons exist for the call, largely due to the possible effects and continuing expansion of oil and gas exploration and extraction within the basin. Despite the debate surrounding the cause of pollution (Wiklund et al. 2012; Kelly et al. 2010), establishing a monitoring program for PAHs in edible portions will provide baseline data in the area so that the status and trends of contamination can be assessed. Furthermore, information on current sources of contaminants such as PAHs are necessary so that appropriate control measures can be implemented. Finally, since PAHs have been naturally released from deposits of bitumen for millennia, it is important to determine the relative proportions emanating due to natural processes and additional releases due to activities of humans, including extraction and upgrading of bitumen.

The aim of this work is to describe the spatial and seasonal distribution of PAHs in muscle of whitefish (Coregonus clupeaformis), northern pike (Exos luscious), walleye (Sander vitreus), goldeye (Hiodon alosoides) and burbot (Lota lota), and to apply a probabilistic approach to estimate risks due to exposure to PAHs through fish consumption in the Athabasca/Slave Rivers (Liang et al. 2013). Inter-season comparison as well as, intra- and inter-specific variability of concentrations of PAHs in muscle of fishes were analyzed at Fort McMurray, Fort McKay, Fort Chipeywan, Fort Smith, and Fort Resolution. Fishes studied were chosen based on their abundance along the basin and their cultural and economic significance to Aboriginal communities. They are therefore of interest in monitoring contaminant levels and assessing the potential for impacts on human health.

3.2 Materials and Methods

3.2.1. Chemicals and Reagents

All solvents used were HPLC grade (Fisher Scientific, Canada). PAH quantification was calibrated using a five point standard calibration curve (2, 10, 40, 200, 800) ng/ml (Wellington Laboratories, Guelph, Canada) containing naphthalene (Nap), acenaphthylene (Acy), acenaphthene (Ace), fluorine (Fl), phenanthrene (P), anthracene (Ant), fluoranthene (Flu), pyrene (Pyr), benzo[a]anthracene (BaA), chrysene (Chr), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[a]pyrene (BaP), indeno[1,2,3-c,d]pyrene (InP), dibenzo[a,h]anthracene (DBA), and benzo[g,h,i]perylene (BgP)]. The calibration standards also contained a 100 ng/ml mixture of isotopically labelled deuterated PAH standards (naphthalene-d8, acenaphthene-d10, fluorene-d10, phenanthrene-d10, anthracene-d10, fluoranthene-d10, pyrene-d10, benz[a]anthracene-d12, chrysene-d12, benzo[b]fluoranthene-d12, benzo[k]fluoranthene-d12, benzo[a]pyrene-d12, indeno[1,2,3-c,d]pyrene-d12, benzo[g,h,i]perylene-d12, dibenz[a,h]anthracene-d14, and dibenzo[a,i]pyrene-d14); and three deuterated PAH internal standards (acenaphthyene-d8, p-terphenyl-d14, benzo[e]pyrene-d12). Recovery standards, containing deuterated PAHs and deuterated PAH internal standards, were also purchased from Wellington Laboratories, Guelph, Canada. The 2000 ng/ml stock solution of the recovery standards was diluted to produce a mixture of 100 ng/ml mixture of surrogate standards. Silica gel (80–100 mesh; Sigma Aldrich, Canada) and anhydrous sodium sulfate (12-60 mesh, Sigma Aldrich, Canada) were baked in a muffle furnace at 450 0C overnight before use. Acid- and base-modified silica were made at ratios of 1:2 (98%) sulphuric acid (EMD, Canada): silica gel and 1:3 (1 N) sodium hydroxide (Sigma-Aldrich, Canada): silica gel, respectively. Modified silicas were then mixed on a roller for 3 h, and used immediately.

3.2.2. Extraction Procedure

For analysis, we optimized US EPA Methods 8270C/D and 1625B. Samples of fish muscle were homogenized and dried with excess anydrous Na_2SO_4. About 15 g wet mass (wm) of fish was then extracted for 18 h in a Soxhlet apparatus with 250 ml dichloromethane (DCM). Deuterated PAHs (naphthalene-d_8, acenaphthene-d_{10}, fluorene-d_{10}, phenanthrene-d_{10}, anthracene-d_{10}, fluoranthene-d_{10}, pyrene-d_{10}, benz[a]anthracene-d_{12}, chrysene-d_{12}, benzo[b]fluoranthene-d_{12}, benzo[k]fluoranthene-d_{12}, benzo[a]pyrene-d_{12}, indeno[1,2,3-c,d]pyrene-d_{12}, benzo[g,h,i]perylene-d_{12}, dibenz[a,h]anthracene-d_{14}, and dibenzo[a,i]pyrene-d_{14}) were added as recovery surrogate standards to all the samples prior to extraction. The soxhlet extract was concentrated to approximately 1–2 ml by rotary evaporation. A mixed bed silica column was used for clean-up. Two grams of basic silica were placed on 1 g of unmodified silica in a glass column (22 cm x 1.5 cm, i.d.). Another 1 g silica was added and then 4 g acid-silica was loaded over the basic-silica layers. The column was then topped with 2 g of anhydrous sodium sulphate. The column was eluted with 30 ml of n-hexane, which was discarded, before the sample was loaded. The fraction containing PAHs was collected by eluting the column with 150 ml of hexane/dichloromethane (1:1). Extract were then concentrated to 1 ml by rotary evaporation, 0.1 ml of nonane containing deuterated PAH internal standards (acenaphthyene-d_8, p-terphenyl-d_{14}, benzo[e]pyrene-d_{12}) was then added to the extract before further concentration to 0.1 ml under a gentle stream of nitrogen.

3.2.3. GC–MS Analysis

PAHs were identified and quantified using a Hewlett Packard (HP) 7890A GC fitted with a 60 m, 0.25 mm i.d., DB-5 silica capillary column and an HP 7683 series autosampler. The injection temperature was 250 ^{0}C and the detector temperature was 280 ^{0}C. The temperature ramp was: 60 ^{0}C for 2 min, 20 ^{0}C/min to 160 ^{0}C followed by 5 ^{0}C/min to 268 ^{0}C and 2 ^{0}C/min to 300 ^{0}C, where it was held for 10 min to give a total run of 55.5 min. The HP 5975 series Mass Selective Detector was operated in Selected Ion Mode (SIM). A 1 µL sample of extract or standard was injected in split/splitless mode. Mass spectra were acquired in electron impact (EI) mode at 70 eV.

3.2.4. Quality Assurance and Quality Control

All analytical data were subject to strict quality control. Method blanks (solvent), and spiked blanks (standards spiked into solvent and reagent) were used to determine background contamination. Some samples were analyzed in duplicate. Instruments were calibrated frequently with certified standards. PAHs were quantified using the internal calibration method based on five-point calibration curves for individual compounds. The surrogate recoveries averaged 84 $\pm$ 16%. Instrument detection limits ranged from 0.1- 2.0 ng/g, wet mass (wm).

3.2.5 Dietary Exposure Estimates

Because health risk criteria are not available for all the individual PAH compounds, the potential carcinogenic risk of PAH mixtures is often expressed using a Toxicity Equivalent Factor approach, This was done by relating the potencies of individual PAHs to that of benzo(a)pyrene (B(a)P, which has the greatest potency of the PAHs to cause cancer (Agency for

Toxic Substances and Disease Registry (ATSDR), 1996). Toxicity equivalence factors (TEFs) relative to B[a]P have been developed for assessing risks posed by mixtures of PAHs (Table 3.1) (Nisbet and LaGoy, 1992). These TEFs were adopted to calculate the potential toxicity of the PAH mixtures measured in this study as total benzo[a]pyrene equivalents ($B[a]P_{eq}$). This approach has been suggested to be superior for assessing the carcinogenic potency of PAH mixtures (Binelli and Provini, 2004; Xia et al., 2010).

Methods for assessment of risks advocated by both Health Canada and the US EPA were used for assessing the carcinogenic risk to humans in the Athabasca/Slave river system due to the consumption of PAHs in fish. To evaluate the potential impacts on the inhabitants of Fort McMurray, Fort McKay, Fort Chipewyan, Fort Smith, and Fort Resolution, it was deemed most appropriate to use Canadian population data, based on local populations, who repeatedly consume fish from the same aquatic source, most of their lives. This approach is more focused on local conditions and customs. The per-capita consumption of fish to estimate the contribution of PAH to the daily intake (DI) is needed. In this regard, since there are no measured data for fish consumption in the Athabasca/Slave Rivers, estimations were used.

Table 3.1: PAHs and their toxic equivalent factors (TEFs) (Nisbet and LaGoy, 1992) adopted to calculate the potential toxicity of the PAH mixtures measured in this study as total benzo[a]pyrene equivalents ($B[a]P_{eq}$).

PAHs	TEFs
Naphthalene (NAP)	0.001
Acenaphthene (ACE)	0.001
Acenaphthylene (ACY)	0.001
Fluorine (FLO)	0.001
Phenanthrene (PHE)	0.001
Anthracene (ANT)	0.01
Fluoranthene (FLA)	0.001
Pyrene (PYR)	0.001
Benz(a)anthracene (BaA)	0.1
Chrysene (CHR)	0.01
Benzo(b)fluoranthene (BbF)	0.1
Benzo(k)fluoranthene (BkF)	0.1
Benzo(a)pyrene (BaP)	1
Dibenz(a,h)anthracene (DahA)	5
Indeno(1,2,3-cd)pyrene (IcdP)	0.1
Benzo(g,h,i)perylene (Bghip)	0.01

The most precise and reliable data on consumption and body weight by various Aboriginal groups in Canada was used (Richardson, 2013; Richardson, 1997) (Appendix B.1&2). In addition, a range of fish consumption rates and representative body mass were used to monitor the potential risk to fish consumers in the sampled locations (Tab 3.2a&b).

Both the concentrations of BaP_{eq} in fishes and the potential daily intake (DI) of PAHs via consumption of fish for specific populations were estimated (Equations 1 and 2),

$$BEC_i = \sum_{i=1}^{n} Ci \text{ x } TEF_i \quad (1)$$

$$DI = \sum_{i=1}^{n} BECi \text{ x } FC \quad (2)$$

Where BECi is the concentration of B[a]P_{eq} in fish (ng/g, wm); and C_i is the concentration of PAH congener i in fish; TEFi = TEF of PAH congener i (Table 1) (Nisbet and LaGoy 1992). FC is fish consumption per day (g/d).

The lifetime cancer risk (LCR) of population groups in the Athabasca and Slave Rivers caused by exposure due to fish consumption was calculated (Equation 3).

$$LCR = \frac{DI \text{ x } ED \text{ x } EF}{BM \text{ x } AT} \; x \; CF \; x \; SFB[a]P \quad (3)$$

Where DI is the daily intake of PAHs via fish consumption (ng/g); ED is duration of exposure (years); EF is the exposure frequency (days/year); BM is the average body mass (kg); AT is averaging time (days); CF is the conversion factor (10^{-6} kg/mg); Cancer-causing ability of B[a]P was used in the determination of oral slope factor. The oral slope factor for B[a]P is 4.5, 5.9, 9.0 and 11.7 with a geometric mean of 7.3 $(mg/kg/day)^{-1}$. The human population in the region of interest, from which samples of fish were collected, was divided into 4 groups according to age: children (4 to <12 years), teens (12 to < 20 years), adults (20 to <65 years), and seniors (≥ 65 years) (Table 3.2c).

Table 3.2a: Estimated log-normal probability density functions describing a range of possible fish consumption rates (g/day) for groups in the study locations.

Sex	Children			Teens			Adults			Seniors		
	Low	Medium	High	Low	Medium	High	Low	Medium	High	Low	Medium	High
Female	70	128	220	50	150	300	60	300	600	50	200	500
Male	70	128	220	50	190	350	60	400	800	50	300	700

Table 3.2b: Estimated log-normal probability density functions describing a range of body mass (kg) for groups in the study locations

Sex	Children			Teens			Adults			Seniors		
	Low	Medium	High	Low	Medium	High	Low	Medium	High	Low	Medium	High
Female	20	35	50	30	55	75	50	100	200	50	80	150
Male	15	32	45	40	60	80	60	150	250	60	90	160

Table 3.2c: Parameters used in the incremental lifetime cancer risk assessment. The risk for each group was calculated separately.

Definition	Units	Children	Teens	Adults	Seniors
Exposure Frequency (EF)	days/year	365	365	365	365
Exposure Duration (ED)	year	70	70	70	70
Averaging Time (AT)	days	25550	25550	25550	25550

3.2.6 Data Analyses

Data exploration was applied to search out patterns before statistical analyses. Box-Whiskers plots were used for descriptive statistical analysis (McGill et al., 1978). The distribution of the histograms was asymmetrical: the distribution was highly skewed. As such, the Shapiro-Wilk test was used to test for normality. The null-hypothesis of this test is that the population is normally distributed. In all cases, the p-values were less than the chosen alpha levels. As such the null hypothesis was rejected because there is evidence that the data tested are not from a normally distributed population. Also, additional test was carried out using Anderson-Darling test. The result further confirmed that the data are not from a normally distributed population. Hence, statistical methods which does not assume a normal distribution of the residual data where chosen. The non-parametric equivalent of the one-way analysis of variance is the Kruskal-Wallis test by ranks (among all observations). The null hypothesis of the Kruskal–Wallis test is not a test of the difference in means or medians of groups. Rather, the Kruskal-Wallis test calculates the sum of the ranks for each group. The test statistic, H, compares variance of the ranks among groups. The P value is the probability of getting a particular value of H by chance if the null hypothesis is true (in order to determine which of the sample pairs are significantly different). Differences in the concentration of PAHs among sampling locations, seasons, and species were evaluated using the Kruskal-Wallis non-parametric test. All statistical analyses were conducted with Microsoft Excel, SigmaPlot for Windows, version 11.0 or Systat for Windows, version 12.0.

3.3 Results

PAHs were detected in all samples of fish muscle collected from the Athabasca and Slave rivers at each location and during each season (Table 3.3). A total of 425 samples of fish muscle among seasons, locations, and species, were analyzed (Table 3.4). The mean concentration of ∑PAHs in muscle of the 425 samples, averaged across species, seasons and locations, was 30 ng/g, wet mass (wm). Mean concentrations among species, locations, and seasons were: ∑2-ring (Naphthalene) 5.8 $\pm$ 1.5 ng/g, wm, ∑3-ring (Acenaphthylene, Acenaphthene, Fluorene, Phenanthrene, and Anthracene) 11 $\pm$ 2.2 ng/g, wm, ∑4-ring (Fluoranthene, Pyrene, Benz(a) anthracene, and Chrysene) 7.2 $\pm$ 2.7 ng/g, wm, ∑5-ring PAHs (Benzo(b) fluoranthene, Benzo(k) fluoranthene, Benzo(a) pyrene, and Dibenz(ah) anthracene) 4.6 $\pm$ 1.4 ng/g, wm, and ∑6-ring (Indeno(1,2,3-cd) pyrene and Benzo(ghi) perylene) PAHs 1.4$\pm$ 0.7 ng/g, wm (Fig 2).

Among all samples, measured concentrations ranged from 1.7 to 81 ng/g, wm for 2-ring PAHs, and from less than the limit of detection (<LOD) to 43 ng/g for 3-ring PAHs, from <LOD to 73 ng/ml for 4-ring PAHs, from <LOD to 26 ng/g for 5-ring PAHs, and from <LOD to 26 ng/g for 6-ring PAHs. The 16 USEPA priority PAHs were observed in muscle from fishes collected at all sampling locations, and were greater in fishes from the AR than from the SR. Concentrations of ∑PAHs measured in fish muscle of the Athabasca/Slave Rivers are similar to those found in other oil producing areas (Nkpaa et al., 2013; Al-Yakoob et al., 1994). Concentrations of ∑PAHs in muscle were not significantly greater than those measured in fishes from non-oil producing areas (Ramalhosa et al. 2012).

Tables 3.3: Mean concentrations (± SD) of 16 parent PAH values in muscles of fish collected at Fort Resolution, Fort Smith, Fort Chipewyan, Fort McKay and Fort McMurray in a) summer 2011, b) fall 2011 and c) spring 2012. : Nap-naphthalenes; Acy-Acenaphthene; Ace- Acenaphthylene; Flu-fluorenes; Phe-phenanthrenes; Ant-anthracenes; Flua-fluoranthenes; Pyr-pyrene; BaA-Benz(a)anthracene; Chr-Chrysene; BbF-Benzo(b)fluoranthene; BkF-Benzo(k)fluoranthene; BaP-Benzo(a)pyrene; DahA-Dibenz(a,h)anthracene; IcdP-Indeno(1,2,3-cd)pyrene; Bghip-Benzo(g,h,i)perylene. Actual numbers ± Standard deviation. n.a.= no specimens available at this location/season. n.d.= bellow detection limit. All values are in ng/g, wet mass (wm).

A.) Summer 2011

Species	Site	Nap	Acy	Ace	Flu	Phe	Ant	Flua	Pyr	BaA	Chr	BbF	BkF	BaP	DbahA	Ind	BghiP
Burbot	FR	n.d	n.d	1.4 ±2.8	1.0 ±2.0	n.d	n.d	0.3 ±0.2	0.5 ±0.5	nd	nd	0.2 ±0.3	ND	0.2 ±0.3	1.0 ±2.6	0.6 ±1.1	0.2 ±0.4
	FS	n.d	n.d	2.0 ±3.8	3.3 ±6.6	n.d	0.9 ±1.9	0.4 ±0.4	0.2 ±0.2	0.2 ±0.2	0.2 ±0.1	n.d	n.d	0.1 ±0.2	0.2 ±0.3	0.2 ±0.3	0.1 ±0.1
	FC	5.9 ±0.5	2.3 ±2.0	n.d	n.d	n.d	n.d	0.4 ±0.1	0.2 ±0.3	0.2 ±0.3	0.15 ±0.1	0.14 ±0.1	0.2 ±0.0	1.36 ±0.0	n.d	n.d	n.d
	FM	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a

	FMU	5.9 ±0.5	2.3 ±2.0	n.d	n.d	n.d	n.d	0.5 ±0.6	0.7 ±1.1	0.1 ±0.2	n.d	0.2 ±0.1	n.d	0.6 ±0.7	0.2 ±0.1	n.d	n.d
Goldeye	FR	11 ±8.5	4.7 ±3.8	7.9 ±5.8	1.3 ±1.4	n.d	n.d	n.d	n.d	0.14 ±0.1	0.18 ±0.2	3.36 ±4.7	2.6 ±3.6	0.7 ±0.4	n.d	n.d	n.d
	FS	n.d	n.d	n.d	n.d	n.d	0.2 ±0.2	0.5 ±0.6	0.3 ±0.3	0.8 ±1.8	0.4 ±0.8	0.1 ±01	n.d	n.d	n.d	0.3 ±0.4	n.d
	FC	1.9 ±1.5	2.3 ±2.1	1.1 ±0.8	n.d	n.d	1.1 ±1.4	1.0 ±1.0	1.5 ±1.7	1.8 ±2.9	0.5 ±0.4	0.5 ±0.4	0.3 ±0.5	1.1 ±1.4	n.d	0.3 ±0.4	0.1 ±0.1
	FM	2.5 ±1.9	2.5 ±1.3	4.3 ±7.2	3.0 ±5.1	1.0 ±1.3	2.3 ±3.4	1.0 ±0.9	3.9 ±8.1	2.2 ±3.8	0.8 ±0.6	0.9 ±1.0	0.7 ±0.6	1.3 ±1.2	1.5 ±2.0	1.1 ±1.3	0.5 ±1.2
	FMU	4.5 ±5.4	9.9 ±17	4.6 ±4.4	5.9 ±9.1	1.9 ±1.8	3.7 ±4.6	1.6 ±2.2	5.2 ±13	2.8 ±3.5	1.4 ±2.1	1.4 ±0.9	1.2 ±0.9	2.7 ±2.2	1.7 ±2.8	1.0 ±2.1	0.4 ±0.5
Jackfish	FR	n.d	n.d	n.d	n.d	n.d	n.d	0.2 ±0.2	0.2 ±0.2	0.5 ±1.1	0.3 ±0.7	0.3 ±0.5	0.2 ±0.4	0.6 ±1.5	0.3 ±0.6	0.2 ±0.3	0.07 ±0.1
	FS	1.7 ±2.2	1.0 ±0.4	0.8 ±0.8	1.2 ±1.6	0.5 ±0.7	0.7 ±1.4	n.d	0.1 ±0.1	1.3 ± 2.4	0.6 ±1.5	0.1 ±0.1	0.2 ±0.3	0.3 ±0.5	0.3 ±0.6	0.2 ±0.3	.2 ±0.3
	FC	2.1 ±1.5	4.8 ±3.6	1.4 ±0.9	n.d	n.d	n.d	0.4 ±0.6	1.0 ±1.4	03 ±0.3	0.1 ±0.1	0.1 ±0.3	n.d	0.1 ±0.1	01 ±0.1	0.2 ±0.5	0.1 ±01
	FM	7.6 ±8.9	4.2 ±4.4	2.9 ±1.5	2.8 ±4.3	2.0 ±3.0	1.6 ±1.7	0.8 ±0.7	1.6 ±2.5	1.7 ±2.5	1.7 ±2.2	1.1 ±1.3	1.0 ±1.0	0.3 ±0.4	2.0 ±4.1	3.2 ±4.2	0.6 ±0.8
	FMU	14.4 ±25	13.4 ±16	7.7 ±11	6.0 ±12	2.3 ±4.0	4.7 ±6.6	1.0 ±0.9	4.4 ±4.4	0.9 ±1.1	0.4 ±0.3	0.5 ±0.4	0.4 ±0.4	1.2 ±0.9	08 ±2.1	0.1 ±0.1	0.2 ±0.2

Walleye	FR	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a
	FS	n.d	n.d	n.d	n.d	n.d	0.3 ±0.4	0.7 ±0.6	0.8 ±0.9	0.8 ±0.9	0.3 ±0.3	0.1 ±0.1	0.1 ±0.1	0.2 ±.3	0.1 ±0.1	n.d	0.1 ±0.2
	FC	2.8 ±2.2	3.4 ±3.4	1.7 ±1.2	n.d	n.d	0.8 ±1.1	2.1 ±2.7	12.2 ±10	0.6 ±0.6	0.5 ±0.5	0.4 ±0.4	0.4 ±0.6	0.6 ±0.3	0.3 ±0.3	0.6 ±0.1	0.2 ±0.4
	FM	8.2 ±6.8	5.3 ±4.1	4.1 ±2.9	4.2 ±7.9	2.4 ±2.4	3.2 ±6.0	3.9 ±53	5.0 ±4.6	2.9 ±3.2	0.9 ±1.0	1.7 ±2.1	0.9 ±1.3	0.7 ±0.9	0.2 ±0.3	0.3 ±0.4	0.3 ±0.4
	FMU	4.3 ±3.4	4.9 ±5.9	3.6 ±4.7	1.8 ±1.6	0.7 ±0.7	1.7 ±1.9	1.5 ±1.6	2.3 ±3.3	1.8 ±2.3	1.1 ±1.5	1.1 ±0.9	0.6 ±0.4	0.2 ±0.3	0.9 ±2.0	0.5 ±0.7	0.4 ±0.5
White fish	FR	n.d	n.d	n.d	n.d	n.d	n.d	0.3 ±0.5	0.6 ±0.7	0.5 ±0.7	0.2 ±0.4	0.1 ±0.1	n.d	0.1 ±0.1	0.2 ±0.5	0.1 ±0.2	n.d
	FS	n.d	n.d	1.8 ±3.1	1.0 ±2.2	n.d	1.0 ±1.5	0.3 ±0.3	0.2 ±0.3	1.8 ±2.8	1.1 ±2.0	n.d	0.2 ±0.3	0.1 ±0.2	0.2 ±0.3	0.3 ±0.6	0.1 ±0.2
	FC	3.3 ±2.8	3.1 ±2.6	3.6 ±4.2	2.7 ±4.5	0.5 ±0.7	0.6 ±0.6	0.7 ±0.6	5.6 ±5.9	1.6 ±1.3	0.8 ±0.5	2.3 ±4.5	1.3 ±1.9	4.7 ±8.6	0.6 ±0.9	0.8 ±0.9	0.2 ±0.2
	FM	4.2 ±3.3	5.3 ±3.6	8.4 ±13	4.2 ±4.7	3.0 ±3.4	1.7 ±2.3	1.7 ±1.8	4.5 ±7.8	6.6 ±11	5.6 ±7.5	3.4 ±3.6	2.1 ±3.3	3.9 ±4.7	2.6 ±2.5	3.5 ±3.8	1.6 ±3.2
	FMU	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a

B.) Fall 2011

Species	Site	Nap	Acy	Ace	Flu	Phe	Ant	Flua	Pyr	BaA	Chr	BbF	BkF	BaP	Dbah A	Ind	Bghi P
Burbot	FR	2.6 ±1.7	1.5 ±1.3	1.7 ±1.4	2.1 ±1.3	3.3 ±2.6	3.7 ±4.7	1.4 ±1.3	0.7 ±0.6	3.4 ±1.3	1.8 ±0.9	0.9 ±0.5	1.1 ±1.1	1.0 ±0.9	1.2 ±1.3	0.8 ±0.6	0.8 ±0.6
	FS	3.1 ±1.0	1.7 ±0.5	3.1 ±0.2	1.9 ±0.0	11.8 ±8.2	14.1 ±6.4	0.5 ±0.4	0.5 ±0.4	6.3 ±8.2	4.1 ±3.0	2.1 ±1.4	1.1 ±1.6	1.9 ±0.7	2.1 ±3.0	n.d	0.2 ±0.3
	FC	5.2 ±1.6	2.3 ±0.5	3.7 ±1.0	1.8 ±0.2	2.4 ±0.9	5.6 ±0.8	1.9 ±0.1	2.6 ±0.4	5.5 ±1.6	2.7 ±1.0	4.8 ±0.8	3.1 ±0.4	1.6 ±0.4	2.4 ±0.4	n.d	1.2 ±0.9
	FM	3.0 ±0.7	1.8 ±1.1	2.2 ±2.1	2.4 ±.2	1.5 ±0.6	2.4 ±1.4	0.9 ±0.2	1.9 ±2.1	3.7 ±2.6	1.3 ±0.4	4.1 ±3.0	2.8 ±1.9	3.6 ±0.0	3.5 ±4.7	2.1 ±2.3	0.4 ±0.4
	FMU	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a
Goldeye	FR	1.1 ±0.7	2.0 ±1.6	0.9 ±0.7	0.9 ±1.1	n.d	0.7 ±0.4	0.4 ±0.4	1.4 ±1.9	0.4 ±0.5	0.5 ±0.4	2.1 ±3.3	1.7 ±2.7	0.6 ±0.3	3.1 ±5.3	2.1 ±1.6	0.3 ±0.3
	FS	2.1 ±1.3	3.0 ±2.5	1.0 ±0.7	n.d	n.d	1.8 ±2.8	1.5 ±1.8	0.2 ±0.2	8.9 ±6.0	4.4 ±3.5	1.9 ±1.4	0.9 ±0.4	0.7 ±0.4	5.8 ±4.6	0.4 ±0.3	0.9 ±0.9
	FC	3.2 ±1.6	1.8 ±1.3	3.4 ±3.2	1.4 ±0.5	1.6 ±0.7	2.3 ±2.7	0.6 ±0.7	1.4 ±1.4	3.5 ±2.1	0.5 ±0.4	1.4 ±1.8	1.2 ±1.3	0.6 ±0.4	0.4 ±0.4	0.4 ±0.4	0.3 ±0.3
	FM	3.9 ±3.8	2.8 ±2.9	1.8 ±1.9	1.6 ±2.4	1.6 ±1.4	1.8 ±1.5	0.6 ±0.7	1.9 ±2.7	4.7 ±5.7	3.1 ±3.7	2.2 ±1.9	2.2 ±1.5	2.6 ±3.8	2.7 ±5.8	2.3 ±2.7	0.7 ±1.0
	FMU	10.3	1.5	n.d	n.d	n.d	n.d	n.d	n.d	n.d	0.2	0.1	0.1	0.9	n.d	n.d	n.d
Jackfish	FR	n.d	1.5 ±0.9	n.d	n.d	n.d	n.d	0.4 ±0.7	n.d	1.0 ±1.0	0.5 ±0.3	0.4 ±0.6	0.4 ±0.4	0.3 ±0.3	0.6 ±0.6	1.1 ±1.0	0.5 ±0.4
	FS	n.d	2.1 ±0.8	n.d	1.0 ±1.5	n.d	n.d	0.5 ±1.2	0.3 ±0.8	0.5 ±1.2	1.1 ±2.1	0.8 ±1.3	1.7 ±2.6	0.3 ±0.3	0.8 ±1.4	1.2 ±1.2	1.2 ±1.6
	FC	5.1 ±4.6	2.6 ±1.9	2.4 ±3.6	2.8 ±5.7	n.d	0.8 ±1.6	0.2 ±0.2	0.2 ±0.3	0.1 ±0.1	0.1 ±0.1	0.3 ±0.3	0.3 ±0.3	0.6 ±0.6	n.d	n.d	0.1 ±0.1
	FM	8.5 ±6.2	4.9 ±7.7	11.5 ±14.5	6.6 ±6.9	3.3 ±4.7	3.9 ±2.1	4.0 ±4.9	3.6 ±2.0	3.0 ±3.4	2.1 ±2.7	0.7 ±0.6	0.8 ±0.6	3.3 ±3.2	2.1 ±3.3	2.3 ±3.4	0.6 ±0.6

	FMU	n.d	2.9 ±2.2	2.2 ±2.1	n.d	1.1 ±0.2	1.0 ±0.0	1.0 ±1.2	0.3 ±0.1	5.2 ±1.1	2.6 ±0.1	0.3 ±0.2	0.3 ±0.0	0.9 ±1.1	0.9 ±0.8	1.3 ±1.3	0.6 ±0.6
Walleye	FR	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	1.03 ±1.9	0.4 ±0.1	0.2 ±0.2	n.d	0.2 ±0.2	0.2 ±0.3	1.3 ±1.1	0.9 ±0.8
	FS	1.6 ±1.7	1.2 ±0.5	1.9 ±1.9	2.1 ±1.6	5.0 ±4.2	5.5 ±5.5	0.3 ±0.3	0.4 ±0.5	5.1 ±3.9	2.3 ±2.2	3.2 ±2.6	2.2 ±1.7	2.1 ±2.1	2.3 ±2.7	1.9 ±2.0	1.4 ±2.0
	FC	n.d	1.7 ±1.0	1.1 ±0.7	0.9 ±0.4	1.6 ±1.9	1.7 ±1.9	0.4 ±0.3	0.4 ±0.3	1.5 ±0.9	0.9 ±0.8	1.3 ±0.7	0.7 ±0.6	1.5 ±1.6	2.8 ±2.9	2.1 ±1.9	1.0 ±0.3
	FM	3.8 ±2.8	3.4 ±3.0	2.8 ±2.5	3.4 ±3.2	3.6 ±3.1	3.4 ±4.3	1.5 ±1.6	1.4 ±1.7	3.9 ±3.9	2.5 ±3.2	0.9 ±1.2	1.1 ±1.1	1.0 ±0.8	1.3 ±1.7	3.1 ±2.4	0.7 ±0.9
	FMU	1.8 ±0.2	6.6 ±3.3	3.7 ±2.3	3.6 ±2.3	2.5 ±2.0	5.2 ±3.0	0.7 ±0.2	0.7 ±0.6	0.4 ±0.0	0.5 ±0.5	0.6 ±0.6	0.5 ±0.5	1.0 ±1.0	1.5 ±1.7	3.6 ±4.4	2.8 ±2.1
White fish	FR	2.4 ±1.7	1.5 ±0.9	1.5 ±1.5	2.8 ±3.8	0.5 ±0.6	0.6 ±0.5	0.4 ±0.5	0.1 ±0.1	1.6 ±1.7	1.0 ±1.3	0.6 ±0.9	0.8 ±1.3	0.6 ±0.5	1.2 ±2.1	0.8 ±0.7	0.9 ±1.5
	FS	n.d	n.d	1.6 ±1.7	1.3 ±1.5	3.9 ±4.6	4.9 ±5.9	0.7 ±1.1	0.5 ±1.1	4.0 ±4.7	1.3 ±1.2	0.2 ±0.2	0.1 ±0.1	0.4 ±0.4	0.1 ±0.1	0.7 ±1.1	0.4 ±0.4
	FC	2.3 ±1.8	1.9 ±1.6	2.4 ±3.2	1.1 ±0.9	0.9 ±1.1	1.3 ±2.1	1.0 ±0.9	3.0 ±3.3	3.5 ±3.4	2.3 ±2.1	1.1 ±0.7	1.4 ±0.9	3.3 ±6.6	1.9 ±2.1	5.1 ±8.2	0.6 ±0.6
	FM	4.9 ±4.9	3.3 ±3.6	3.2 ±3.4	3.4 ±3.2	2.0 ±1.7	4.3 ±3.5	2.6 ±3.5	2.6 ±3.9	4.0 ±6.6	3.3 ±5.3	2.6 ±2.6	1.9 ±1.9	3.4 ±7.1	0.9 ±1.2	1.8 ±2.2	0.5 ±0.6
	FMU	2.9 ±3.4	2.2 ±2.2	1.4 ±1.1	3.5 ±4.5	1.7 ±2.3	3.2 ±6.4	2.1 ±3.0	2.8 ±3.6	2.3 ±2.1	2.6 ±2.4	1.5 ±1.5	1.3 ±1.4	1.1 ±1.3	1.0 ±1.6	0.6 ±0.8	0.5 ±0.7

C.) Spring 2012

Species	Site	Nap	Acy	Ace	Flu	Phe	Ant	Flua	Pyr	BaA	Chr	BbF	BkF	BaP	DbahA	Ind	BghiP
Burbot	FR	1.2 ±0.7	1.1 ±0.6	2.3 ±2.0	1.0 ±1.3	0.9 ±1.4	1.0 ±1.4	n.d	1.3 ±2.3	n.d	n.d	0.1 ±01	0.1 ±0.1	0.1 ±0.0	n.d	n.d	0.1 ±0.0
	FS	n.d	n.d	n.d	0.9	n.d	n.d	n.d	0.1	0.3	0.3	n.d	n.d	0.1	0.5	1.0	n.d
	FC	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a
	FM	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a
	FMU	25.0 ±21.4	2.5 ±0.7	1.2 ±0.1	5.3 ±1.9	1.8 ±1.1	2.9 ±1.5	4.3 ±1.9	2.0 ±1.2	36.4 ±32.0	22.3 ±13.0	6.8 ±3.9	2.9 ±3.3	1.2 ±1.0	n.d	0.3 ±0.2	1.5 ±0.9
Goldeye	FR	1.3 ±0.8	1.0 ±0.4	0.8 ±0.6	0.7 ±0.3	0.2 ±1.7	0.2 ±2.2	0.5 ±0.5	0.8 ±0.9	0.3 ±0.3	0.1 ±0.1	0.8 ±0.6	0.5 ±0.3	1.1 ±0.9	0.2 ±0.3	1.3 ±1.4	0.1 ±0.2
	FS	n.d	1.6 ±0.3	0.7 ±0.5	n.d	n.d	n.d	n.d	n.d	0.3 ±0.3	0.2 ±0.2	0.8 ±1.5	0.6 ±1.0	0.2 ±0.2	0.3 ±0.2	0.5 ±0.3	0.9 ±0.9
	FC	n.d	n.d	1.1 ±1.7	n.d	n.d	n.d	n.d	0.8 ±1.5	0.3 ±0.1	0.2 ±0.1	0.2 ±0.3	0.2 ±0.3	0.4 ±0.5	2.5 ±3.8	1.5 ±2.5	0.2 ±0.4
	FM	20.9 ±12.6	1.7 ±1.0	2.1 ±1.5	2.9 ±1.9	1.7 ±2.3	1.9 ±1.2	1.5 ±1.8	1.2 ±1.2	5.9 ±4.4	3.8 ±3.3	9.0 ±7.3	5.5 ±3.4	4.8 ±2.6	0.1 ±0.1	0.7 ±0.8	0.7 ±0.8

Jackfish	FMU	23.0 ±15.5	1.8 ±0.7	2.8 ±3.9	4.6 ±4.6	1.8 ±1.0	2.4 ±1.5	0.8 ±0.6	0.8 ±1.2	6.9 ±6.8	3.1 ±3.1	7.2 ±5.0	4.1 ±4.2	3.3 ±4.1	0.5 ±0.8	0.5 ±0.9	0.7 ±0.6
	FR	1.0 ±0.7	1.5 ±0.9	1.4 ±1.2	1.0 ±1.0	0.6 ±0.5	0.4 ±0.5	0.1 ±0.2	0.4 ±1.0	0.1 ±0.1	0.1 ±0.1	0.2 ±0.2	0.2 ±0.1	0.4 ±0.4	0.6 ±1.1	1.2 ±0.8	0.2 ±0.1
	FS	1.8 ±0.7	0.9 ±0.3	1.5 ±1.3	1.6 ±2.5	0.9 ±0.6	0.6 ±0.5	0.3 ±0.3	0.2 ±0.1	0.2 ±0.2	0.2 ±0.1	0.2 ±0.2	0.3 ±0.3	0.1 ±0.1	0.4 ±0.4	0.1 ±0.2	0.1 ±0.1
	FC	4.4 ±3.0	3.5 ±4.5	1.2 ±1.0	1.2 ±1.4	1.0 ±1.6	0.6 ±0.8	0.5 ±1.0	0.1 ±0.1	1.7 ±2.5	0.8 ±1.5	0.3 ±0.3	0.4 ±0.3	0.8 ±1.7	0.8 ±1.7	2.1 ±2.4	0.5 ±0.6
	FM	26.1 ±15.9	2.5 ±0.3	1.7 ±1.0	4.7 ±4.0	3.7 ±1.6	8.3 ±0.8	1.9 ±1.3	0.7 ±0.6	15.1 ±6.1	8.3 ±7.0	2.1 ±2.3	0.9 ±0.5	1.0 ±0.4	0.3 ±0.3	2.1 ±3.8	1.1 ±1.5
Walleye	FMU	35.4 ±17.3	2.3 ±1.2	1.6 ±1.6	4.1 ±2.2	3.3 ±1.3	4.8 ±2.6	3.4 ±1.4	1.2 ±0.6	5.5 ±2.9	3.0 ±1.3	5.3 ±3.7	3.4 ±2.7	1.6 ±0.4	0.4 ±0.3	0.5 ±0.5	1.0 ±0.9
	FR	1.1 ±1.0	1.5 ±0.9	0.9 ±0.7	0.7 ±0.7	0.6 ±0.7	0.6 ±0.7	n.d	0.6 ±0.9	0.5 ±0.8	0.2 ±0.4	0.4 ±0.4	0.5 ±0.5	0.9 ±0.9	0.2 ±0.2	2.1 ±2.1	0.3 ±0.3
	FS	1.0 ±1.0	n.d	1.9 ±1.7	1.7 ±1.7	1.7 ±1.7	n.d	0.4 ±0.1	0.1 ±0.1	0.2 ±0.3	0.4 ±0.8	0.2 ±0.4	0.2 ±0.1	0.3 ±0.3	n.d	0.1 ±0.2	0.1 ±0.1
	FC	2.6 ±2.0	2.3 ±2.1	2.2 ±3.4	1.8 ±3.0	1.8 ±3.3	1.8 ±2.9	0.9 ±0.6	5.1 ±8.9	0.1 ±0.1	0.1 ±0.0	0.4 ±0.2	0.3 ±0.1	0.1 ±0.1	n.d	n.d	0.1 ±0.2
	FM	24.9 ±18.4	1.7 ±0.5	1.5 ±0.9	2.7 ±1.0	1.7 ±1.3	3.6 ±1.0	4.1 ±5.0	1.5 ±1.3	11.2 ±5.1	9.9 ±6.9	8.3 ±8.1	7.4 ±4.7	1.9 ±1.4	0.1 ±0.1	0.3 ±0.3	0.5 ±0.7
	FMU	11.3 ±7.0	2.6 ±1.1	1.2 ±0.9	1.5 ±1.2	2.3 ±1.0	3.1 ±1.5	3.1 ±1.2	1.1 ±0.3	2.5 ±0.4	1.8 ±0.3	3.0 ±1.2	1.6 ±1.4	2.3 ±0.9	n.d	n.d	n.d

White fish	FR	2.0 ±2.7	1.6 ±1.7	2.4 ±3.1	2.2 ±2.7	1.8 ±1.9	1.4 ±1.5	0.2 ±0.3	n.d	0.1 ±0.2	0.1 ±0.1	0.3 ±0.4	0.3 ±0.4	0.4 ±0.5	0.1 ±0.1	0.9 ±0.9	0.2 ±0.3
	FS	1.3 ±1.0	1.4 ±1.4	1.2 ±1.7	0.8 ±1.0	0.1 ±0.1	0.3 ±0.5	0.1 ±0.1	1.8 ±3.0	0.1 ±0.1	0.6 ±0.9	0.3 ±0.3	0.1 ±0.1	0.7 ±0.9	n.d	0.2 ±0.4	0.1 ±0.2
	FC	2.2 ±1.8	0.6 ±0.4	2.3 ±3.1	0.9 ±0.6	0.8 ±0.9	0.6 ±0.5	0.6 ±0.8	1.0 ±1.5	2.4 ±3.1	1.4 ±1.9	0.1 ±0.1	0.1 ±0.2	0.1 ±0.2	0.2 ±0.2	1.5 ±2.2	0.3 ±0.4
	FM	29.3 ±14.5	6.2 ±5.6	18.3 ±21.8	4.9 ±2.8	1.3 ±0.0	7.5 ±9.8	0.3 ±0.3	2.1 ±2.9	1.0 ±1.3	0.8 ±0.9	3.0 ±4.1	0.7 ±0.7	0.7 ±0.5	n.d	0.3 ±0.3	0.5 ±0.6
	FM U	23.0 ±14.8	6.7 ±9.3	9.6 ±16.4	7.1 ±8.6	3.3 ±2.7	6.5 ±10.8	1.9 ±2.1	1.2 ±0.6	8.6 ±7.2	6.0 ±4.6	4.5 ±3.6	2.3 ±2.9	0.8 ±0.6	0.4 ±0.5	0.2 ±0.2	0.5 ±0.7

Table 3.4: Number of fish collected during the sampling period from the sampling locations.

	Fort Resolution	Fort Smith	Fort Chipewyan	Fort McKay	Fort McMurray	Total
Burbot	22	8	5	2	6	43
Goldeye	12	18	16	28	15	89
Jackfish	24	19	20	20	21	104
Walleye	17	23	15	23	18	96
Whitefish	26	18	18	20	11	93
Total	101	86	74	93	71	425

3.3.1 Exposure Associated with Species, Seasons and Locations

Kruskal Walis test was performed to compare within species for all locations and seasons and then among species by location and season (Table 3.5). Significant differences in concentrations of ∑PAHs in muscle were observed for goldeye, jackfish, walleye and whitefish among sites (Table 3.5). Tests to compare within species for seasonal variation only showed significant differences in concentrations of ∑PAHs in muscle of burbot (Test Statistic = 20, p-value = 0.000). There were no significant differences among species by site and by season (Table 3.5). Analysis by season for locations showed statistically significant differences in ∑PAHs within summer (Test Statistic = 87.7, df = 4, p = 0.000), fall (Test Statistic = 24.1, df = 4, p = 0.000), and spring (Test Statistic = 85.9, df = 4, p = 0.000). In general, greater concentrations of ∑PAH were detected in fishes collected from the AR relative to the SR (Fig. 3.1). The concentration of ∑PAHs in muscle of fishes from near Fort McMurray ranged from 11 ng/g, wm (burbot, summer) to 116 ng/g, wm (burbot, spring) with a mean concentration of 48 ng ∑PAHs/g, wm. The concentration of ∑PAHs in muscle of fishes from near Fort McKay ranged from 29 ng/g, wm (goldeye, summer) to 81 ng/g, wm (walleye, spring) with mean value of 53 ng/g, wm. The concentration of ∑PAHs in muscle of fishes from near Fort Chipewyan varied from 11 ng/g, wm (burbot, summer) to 47 ng/g, wm (burbot, fall) with mean value of 22 ng/g, wm. The concentration of ∑PAHs in muscle of fishes from near Fort Smith ranged from 3.8 ng/g, wm (burbot, spring) to 55 ng/g, wm (burbot, fall) with mean concentration of 16 ng/g, wm. While the concentration of ∑PAHs in muscle from fish collected near Fort Resolution ranged from 4.3 ng/g, wm (whitefish, summer) to 33 ng/g, wm (goldeye, summer) with a mean of 13 ng ∑PAHs in muscle/g, wm. The greatest concentration of ∑PAHs was observed in muscle of fishes collected during spring sampling (Fig 3.2).

Table 3.5: Kruskal-Wallis non-parametric one-way analysis of variance test showing the within species statistic and p values for locations and seasons.

	Locations		Seasons	
Species	Statistic	p-Value	Statistic	p-Value
Walleye	41.29	0.000	0.08	0.961
Goldeye	36.45	0.000	4.64	0.098
Jackfish	52.47	0.000	0.36	2.062
Burbot	8.41	0.078	20.00	0.000
Whitefish	37.44	0.000	8.50	0.014

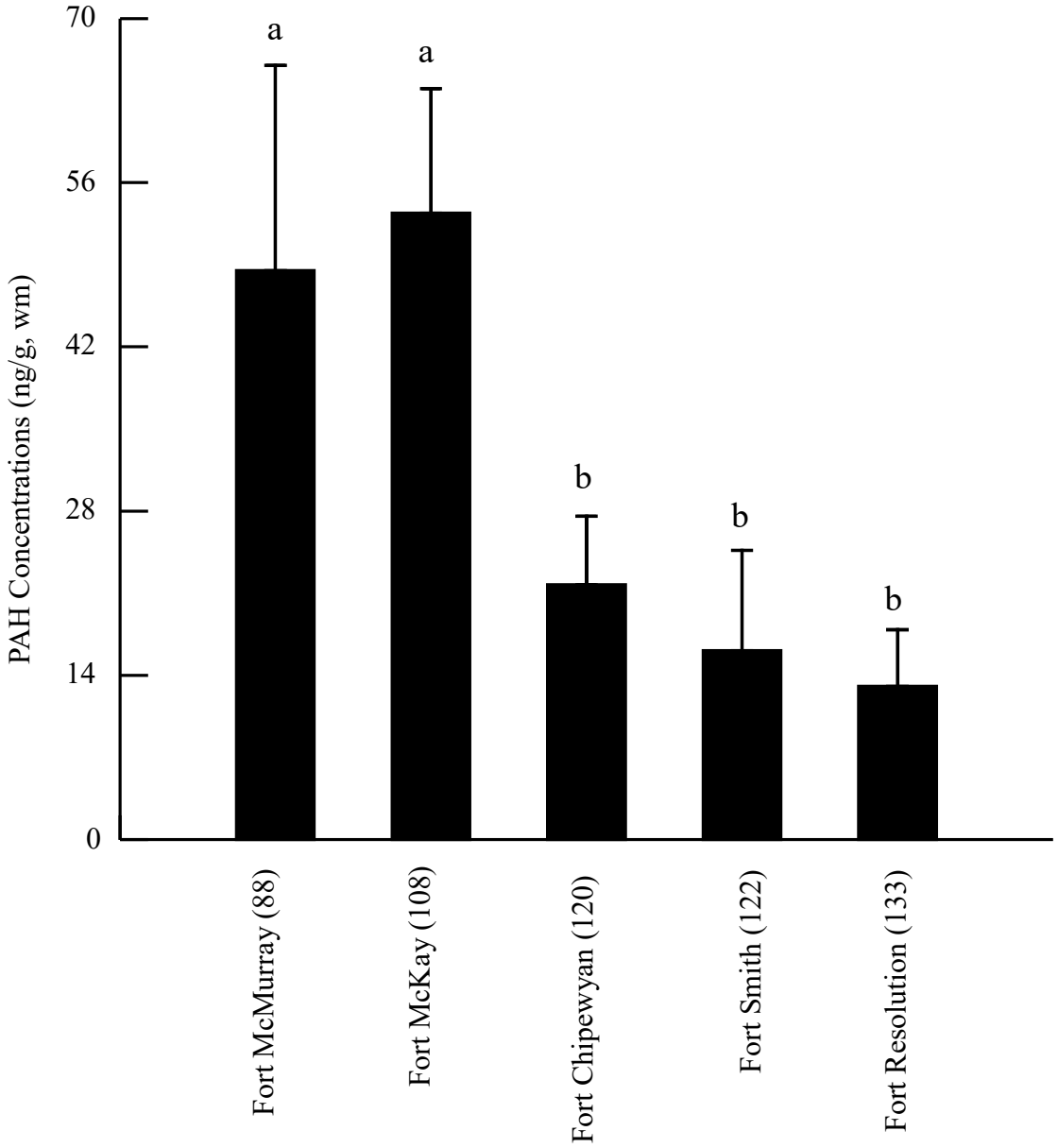


Figure 3.1: Mean concentrations of PAHs (ng/g, wet mass (wm)) and error bars showing variations across sampling locations. Statistical differences between pairs of locations ($p < 0.05$) are indicated by different letters.

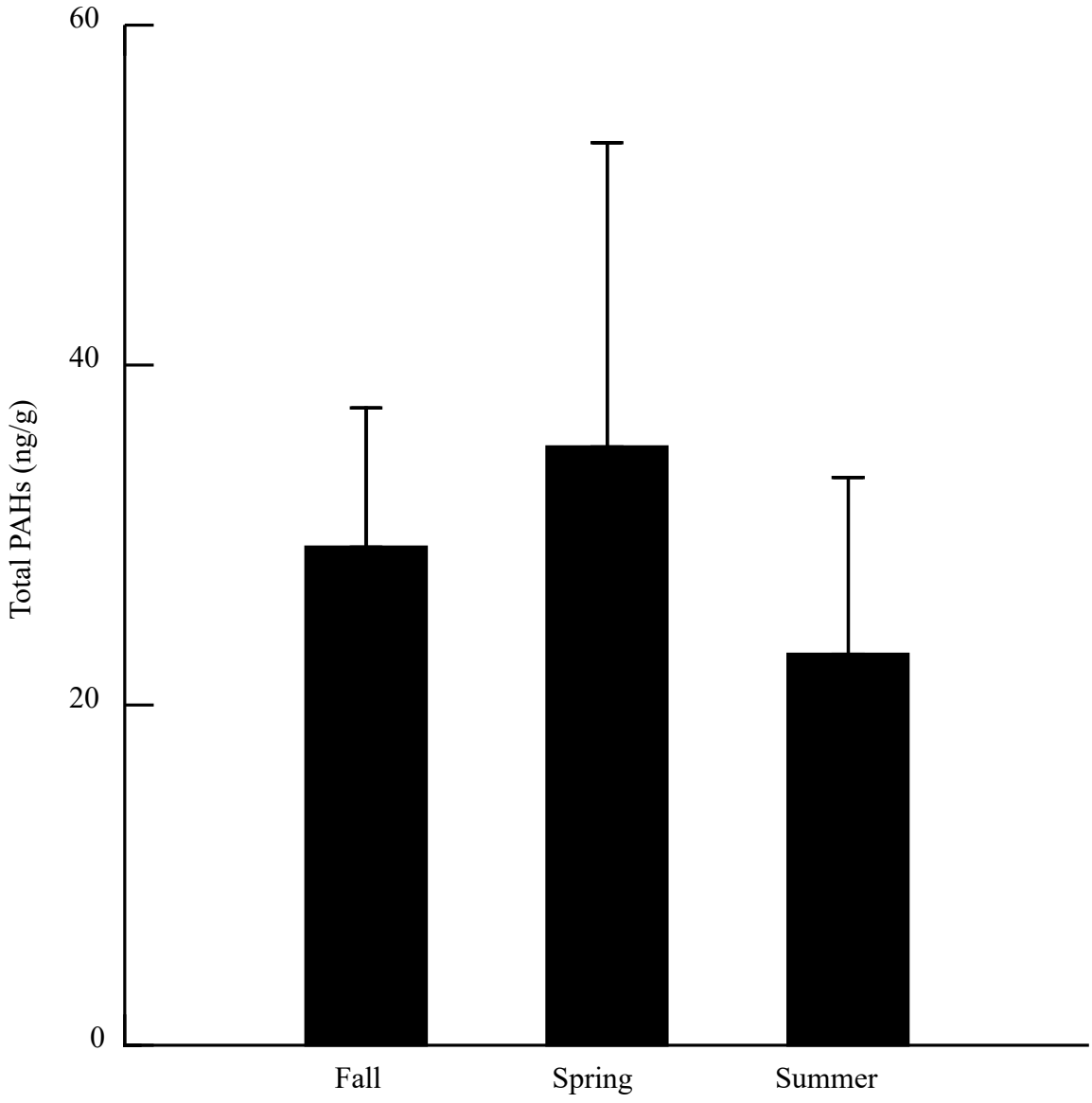


Figure 3.2: Mean concentrations of PAHs (in ng/g wet mass (wm)) and error bars showing variations across sampling seasons. No statistical differences between pairs of seasons ($p > 0.05$).

3.3.2 Concentrations of PAHs in Northern Pike

Since some of the collected species migrate seasonally, they might be exposed to different sources of contaminants during different seasons. In contrast, northern pike (*Esox luscious*; jackfish) rarely travel significant distances, this territorial behavior makes them a more suitable indicator species for localized contamination (Fig. 3.3). Concentrations of ∑PAHs in pike were greatest at Fort McMurray and least at Fort Resolution. Concentrations of ∑PAHs in pike from Fort Resolution ranged from 1.8 ng/g, wm (summer) to 17.2 ng/g, wm (summer) with a mean value of 7.8 ng/g wm. Concentrations of ∑PAHs in pike from Fort Smith ranged from 2.5 ng/g, wm (summer) to 38.8 ng/g, wm (fall) with a mean of 10.8 ng/g, wm. Concentrations of ∑PAHs in pike from Fort Chipewyan ranged from 2.4 ng/g, wm (summer) to 41.7 ng/g, wm (spring) with a mean of 15.9 ng/g, wm. Concentrations of ∑PAHs at Fort McKay ranged from 4.3 ng/g, wm (fall) to 100.2 ng/g, wm (spring) with a mean of 44 ng/g, wm. Concentrations of ∑PAHs at Fort McMurray ranged from 15.6 ng/g, wm (summer) to 241 ng/g, wm (summer) with mean value of 45 ng/g, wm. The results are consistent with previous finding (Chapter 2), there being greater concentrations of PAHs in the AR, relative to the SR.

3.3.3 Human Health Risk Assessment

Risks of adverse effects to humans, associated with PAH exposure can be determined by comparing measurable concentrations to health based regulatory limits. Mean concentrations of BaP_{eq} in various fishes are presented in Table 3.6. The calculated concentration of BaP_{eq} in fish was consistent with the spatial trends in concentrations of PAHs. Nevertheless, Fort Chipewyan, where concentrations of PAHs were less than those at Fort McMurray, had greater carcinogenic potential than that of Fort McMurray (Fig. 3.4). This is because of greater concentrations of

PAHs with larger TEF values, e.g., benzo(a)anthracene and benzo(k)fluoranthene. The least concentration of BaP_{eq} (1.56 ng/g wm) was measured in walleye from Fort Resolution, while the greatest concentration (11.9 ng/g wm) was measured in burbot from Fort McKay.

Table 3.6: Mean concentration of the total PAHs (in ng/g wm) found in collected fish and the Toxicity equivalence factors (TEFs) relative to Benzo[a]pyrene (B[a]P) (TEBaP) values. wm=wet mass; fm = fish mass.

Location	Average PAHs (ng/g wm)	Average PAHs (ng TE BaP/g wm)	Average PAHs (ng/g fm)	Average PAHs (ng TE BaP/g f.m)
Fort Resolution	12.7	3.8	9.3E-3	2.8E-3
Fort Smith	17.2	5.4	14E-3	4.4E-3
Fort Chipewyan	22.2	6.2	16E-3	4.5E-3
Fort McKay	48.7	9.8	41E-3	8.4E-3
Fort McMurray	50.1	5.6	40E-3	4.5E-3

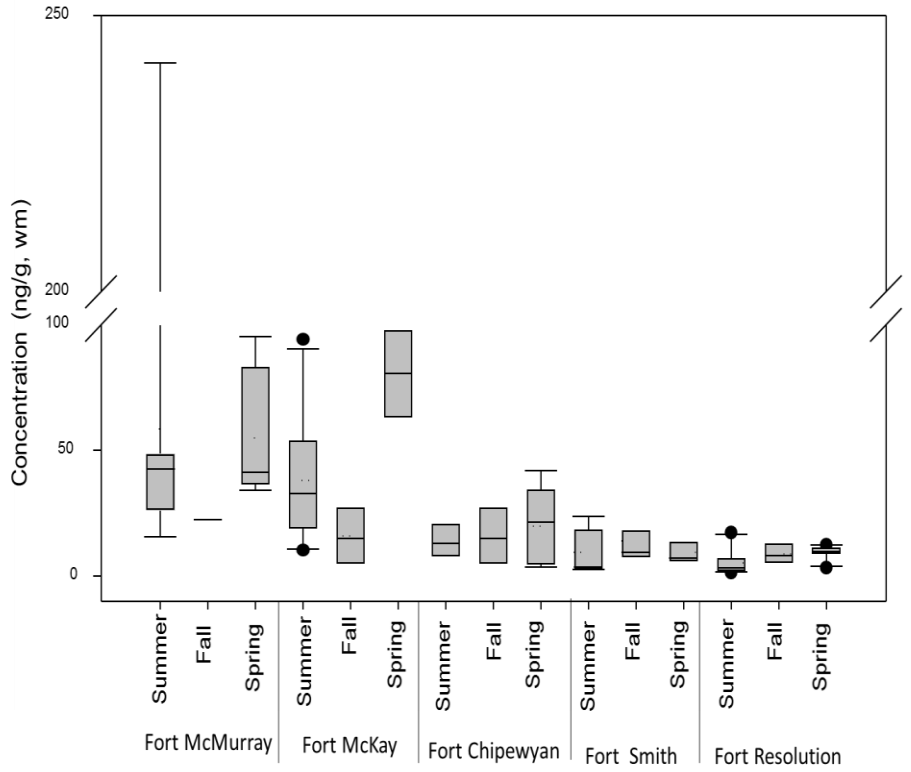


Figure 3.3: Box plots showing the spread of concentrations (ng/g, wm) of PAH levels in muscle of northern pike from the five locations, during three seasons (in ng/g wet mass (wm)). Confidence interval is 95%. Thick line is the median. The width of the box shows the interquartile range. The top 50% of the concentration are represented by everything above the median. The top 25% concentrations are shown by the top whisker.

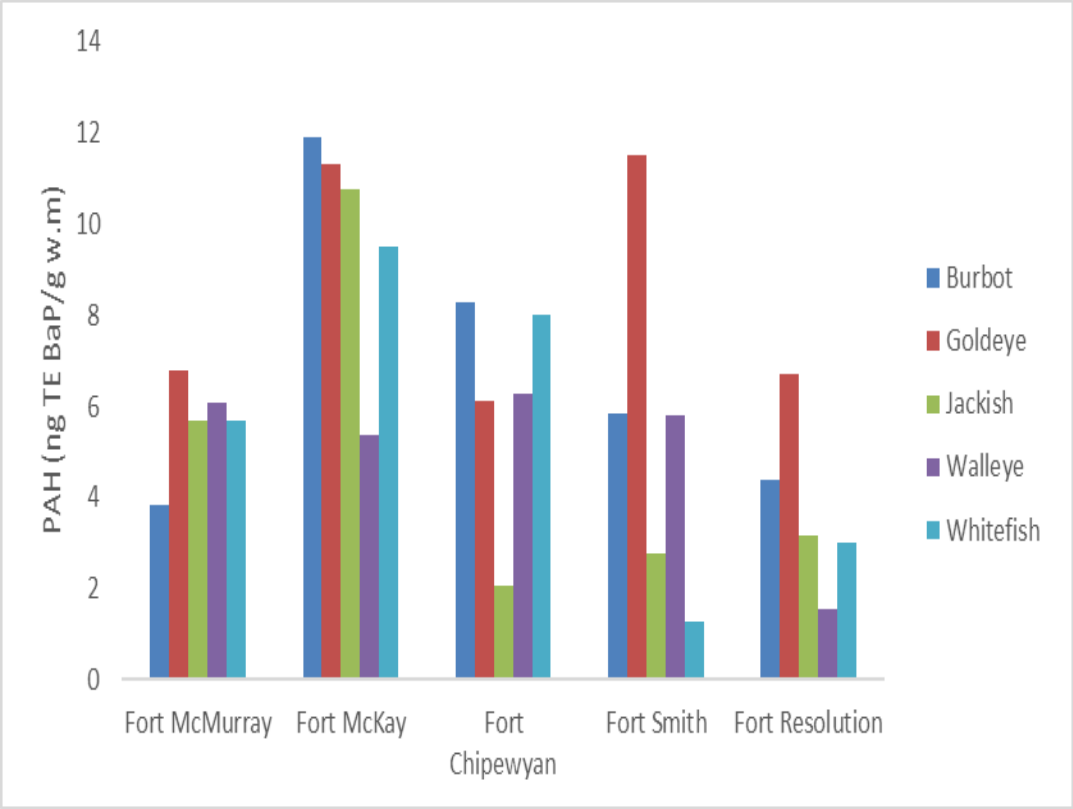


Figure 3.4: The average concentrations of Toxicity equivalence factors relative to Benzo[a]pyrene, $TEBaP_{eq}$ (ng/g wet mass (wm)) values of different fish in sampling locations.

3.3.4 Minimal Risk Levels (MRLs)

To develop an understanding of the potential risk to human health based on PAH intake via fish consumption, it was necessary to evaluate risk based on the most sensitive PAH induced endpoint of relevance to humans. Minimal Risk Levels (MRLs) are reference values to evaluate the toxicity of PAHs based on acute (1-14 days), intermediate (14-365 days), and chronic (365 days and longer) oral exposures (Table 3.7). It was possible to use the daily rate of consumption of fish to calculate an intermediate oral exposure. In this case, possible human exposures were less than MRL values, thus presenting no remarkable risk to humans. For example, the DI of PAHs due to consumption of fish at Fort McMurray was 8% of the MRL for an intermediate exposure. Therefore, it is unlikely that PAHs derived from consumption of fishes in the Athabasca/Slave Rivers would cause intermediate-level adverse effects to humans. Furthermore, the reference value was based on USEPA assumptions of daily consumption of 227 g of fish from the same location over a 70-year life span (USEPA 1991a). Using this consumption value, the estimated daily intakes (DI) for Fort Resolution, Fort Smith, Fort Chipewyan, Fort McKay, and Fort McMurray were 12.2, 17.6, 19.9, 31.7 and 18.2 ng BaP_{eq} /kg body mass (bm) per day, respectively. The result obtained for an acute exposure was even less than that for MRL values by several orders of magnitude. As a conservative approximation, the greatest observed concentration for each species and a range of possible fish consumption rates were used to calculate a DI for each species (Table 3.8). The potential for possible cancer risk due to PAHs from consumption of the most contaminated fish species in the study area is highly unlikely.

Table 3.7: Minimal risk level (MRL) for different PAHs formulated by the Agency for Toxic Substances and Disease Registry (ATSDR) (1996) according to the duration of oral exposure (Agency for Toxic Substances and Disease Registry (ATSDR) 1996).

Compound	Duration	MRL(mg/kg/d)	Factor of uncertainty	Endpoint
Anthracene	Interm.	10	100	Hepatic
Fluoranthene	Interm.	0.4	300	Hepatic
Fluorene	Interm.	0.4	300	Hepatic
Naphthalene	Acute	0.05	1000	Neurol.
	Interm	0.02	300	Hepatic

Table 3.8: Daily Intakes (DI) of PAHs (ng BaP_{eq} /kg body weight per day) at different body masses using the highest observed TEBaP concentration for each species, based on different daily consumption of fish for a) female and b) male groups.

A) Females Groups

Species	Max.TEBaP	Children			Teens			Adults			Seniors		
		Low	Medium	High	Low	Medium	High	Low	Medium	High	Low	Medium	High
Burbot	11.9	6.0	43.5	52.4	19.8	32.5	47.6	14.3	35.7	35.7	11.9	29.8	39.7
Goldeye	11.5	5.8	42.1	50.6	19.2	31.4	46.0	13.8	34.5	34.5	11.5	28.8	38.3
Jackfish	10.8	5.4	39.5	47.5	18.0	29.5	43.2	13.0	32.4	32.4	10.8	27.0	36.0
Walleye	6.3	3.2	23.0	27.7	10.5	17.2	25.2	7.6	18.9	18.9	6.3	15.8	21.0
Whitefish	9.5	4.8	34.7	41.8	15.8	25.9	38.0	11.4	28.5	28.5	9.5	23.8	31.7

B) Males

Species	Max.TEBaP	Children			Teens			Adults			Seniors		
		Low	Medium	High	Low	Medium	High	Low	Medium	High	Low	Medium	High
Burbot	11.9	7.9	47.6	58.2	14.9	37.7	52.1	11.9	31.7	38.1	9.9	39.7	52.1
Goldeye	11.5	7.7	46.0	56.2	14.4	36.4	50.3	11.5	30.7	36.8	9.6	38.3	50.3
Jackfish	10.8	7.2	43.2	52.8	13.5	34.2	47.3	10.8	28.8	34.6	9.0	36.0	47.3
Walleye	6.3	4.2	25.2	30.8	7.9	20.0	27.6	6.3	16.8	20.2	5.3	21.0	27.6
Whitefish	9.5	6.3	38.0	46.4	11.9	30.1	41.6	9.5	25.3	30.4	7.9	31.7	41.6

3.3.5 Potential for Risks to Local Populations

The daily intake (DI) to PAHs due to consumption of fish, for each population group at Fort Resolution, Fort Smith, Fort Chipewyan, Fort McKay and Fort McMurray were calculated (Fig. 3.5a & 3.5b). The median $B[a]P_{eq}$ daily intakes due to fish consumption for male groups were estimated to be 748, 1093, 1285, and 1046 ng/g/d, respectively and that for females at the same locations were 744, 619, 1577, and 1041 ng/g/d for children, teenagers, adults and seniors, respectively. The intake of $B[a]P_{eq}$ increased in the order for males: children, teen, senior and adults. For females, the increasing order was: teens, children, seniors and adults. Based on our estimates, the female adults of Fort McKay have greater potential for exposure (3218 ng/d) to $B[a]P_{eq}$ from consuming fish meals while female teens in Fort Smith have the least exposure (133 ng/d). In general, across all age groups, males were predicted to have slightly greater daily exposure (1097 ng/d) than did females (1051 ngd^{-1}). This result is similar to other studies (Xia et al., 2010; Martí-Cid et al., 2008). We used a wide range of possible fish consumption rates and body mass values (low, medium, high) to calculate possible risks to consumers based on the measurable PAH values in the sampled locations (Table 3.2a&b). None of the values presents appreciable risk to human consumers in the areas. The cumulative probability distributions of the calculated LCR are presented in Table 9. The average values of LCR for all population groups were lower than the range of one in a million (10^{-6}) chance of additional human cancers over a 70-year lifetime (LCR = 10^{-6}).

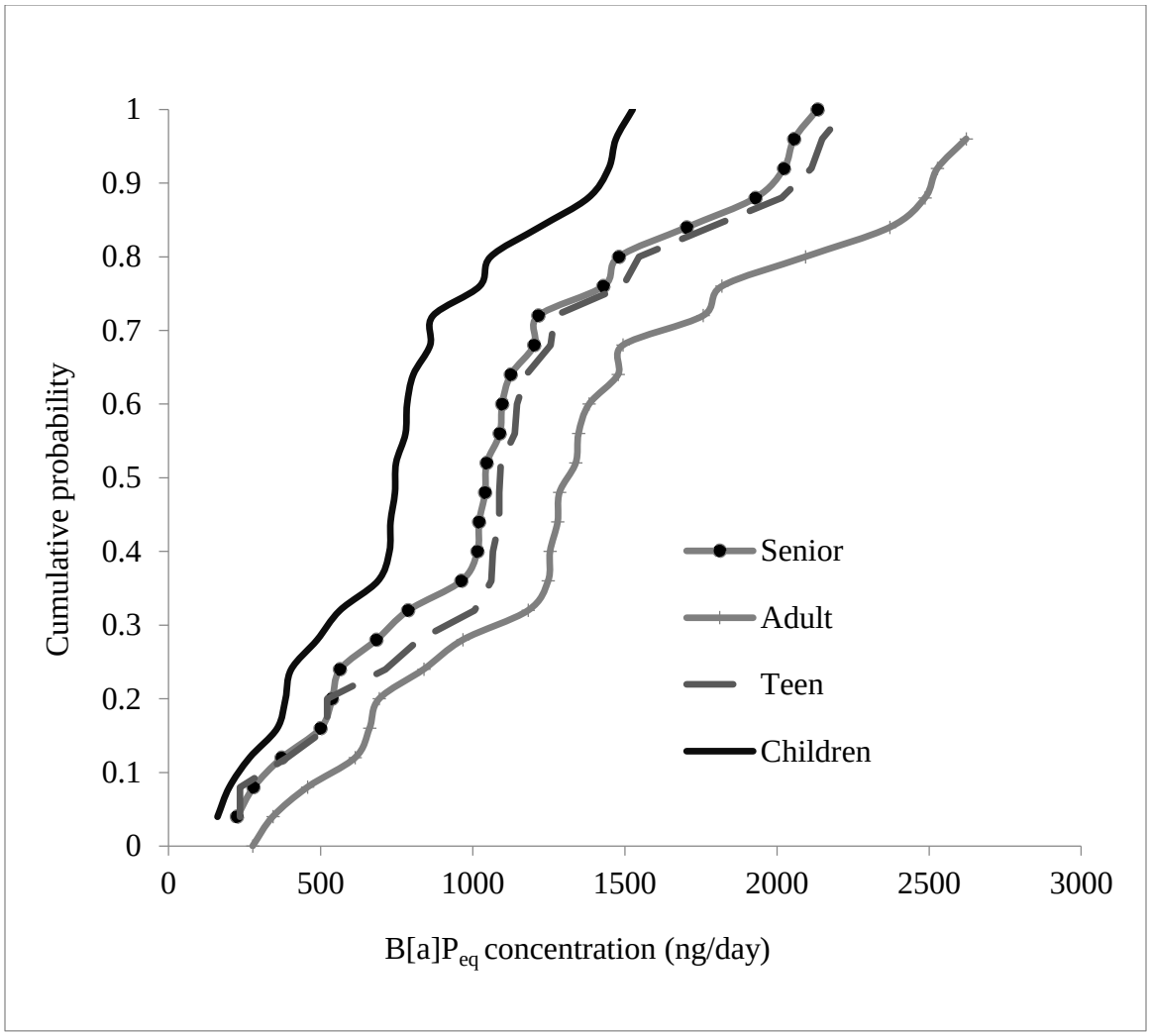


Figure 3.5a: Probability distributions of daily dietary benzo[a]pyrene (B[a]P) equivalent ($B[a]P_{eq}$) exposure for male population groups in Fort Resolution, Fort Smith, Fort Chipewyan, Fort McKay and Fort McMurray.

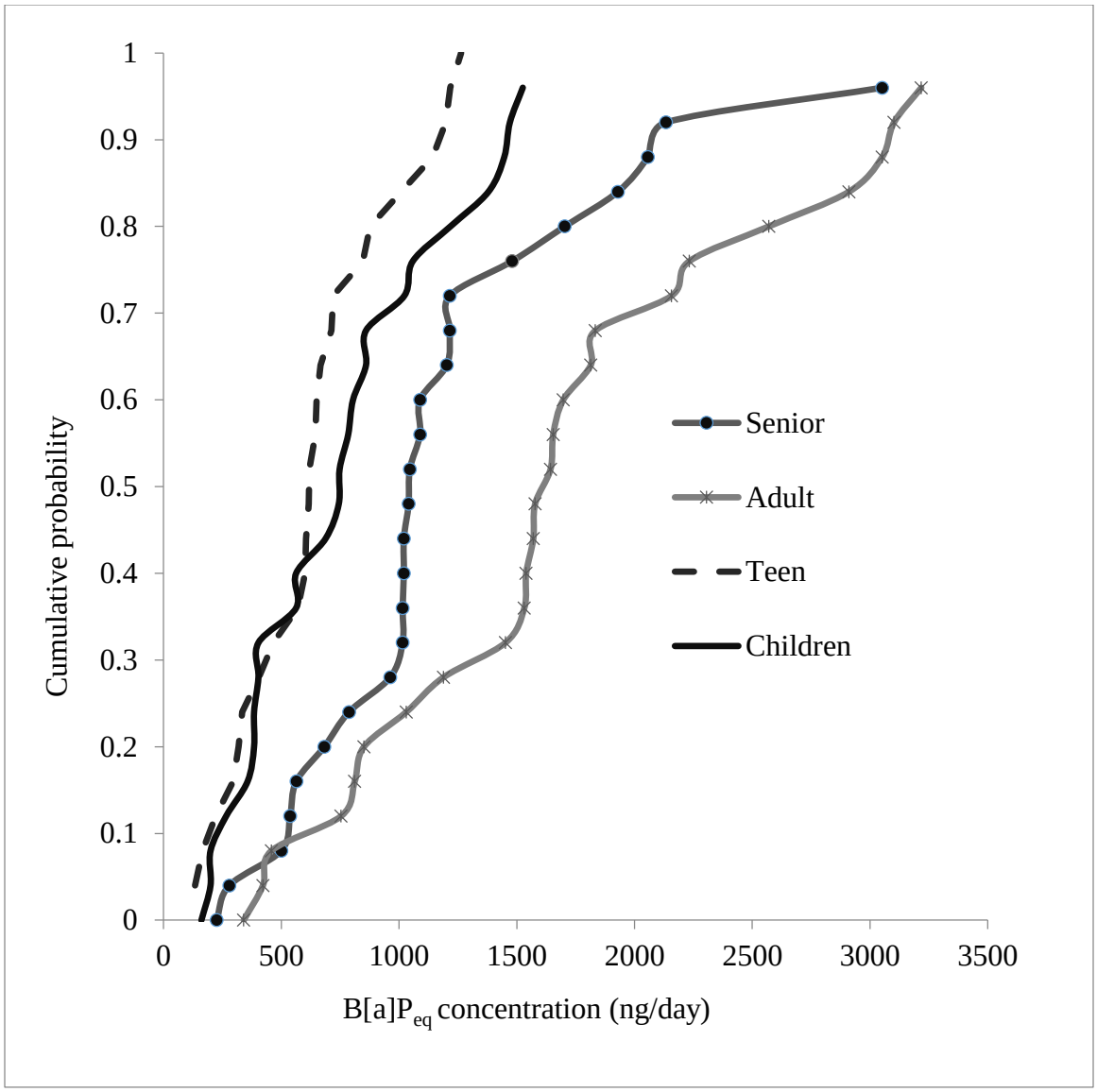


Figure 3.5b: Probability distributions of daily dietary benzo[a]pyrene (B[a]P) equivalent ($B[a]P_{eq}$) exposure for female population groups in Fort Resolution, Fort Smith, Fort Chipewyan, Fort McKay and Fort McMurray.

3.4 Discussion

The relatively small concentrations of individual PAHs observed in the fish muscle tissues are clearly related to the relatively rapid depuration of these contaminants in fish (Ahokas and Pelkonen 1984). Complex phenomena, mainly ecology, such as preferred habitat, and bioavailability of individual compounds influence exposure to PAHs (Simonin et al. 2008). Physical characteristics like temperature, turbidity (Kerkhoven and Gan 2011) and acidity of systems also affect organic contaminant distribution in aquatic biota (Schindler et al. 1995). The Athabasca and Slave Rivers are hard water rivers with relatively great concentrations of mainly bicarbonate salts of calcium. Also, the particulate organic carbon and dissolved organic carbon will influence PAH bioavailability (RAMP 2012).

The fishes studied were assigned to trophic levels ranging from 2 to 4. Lake whitefish is a first order carnivore (Scott, Crossman 1979; Nelson, Paetz 1992). Burbot, walleye and northern pike are piscivores (Braune et al. 1999). Muscle concentrations are greater in lower tropic level species as were concentrations of PAHs in bile (Ohiozebau et al. 2015). This may in part be due to biodiminution between trophic levels. PAHs have relatively short metabolic half-lives in vertebrates, and as such do not show a tendency to biomagnify. They are readily degradable compounds that are subject to metabolic clearance at lower trophic levels, reducing their potential to be passed along food chains.

Whitefish had the greatest concentrations of PAHs of the collected species from all sites and seasons. Due to their lipophilic nature the availability of PAHs decreases in open water relative to the benthic zone, thus affecting bottom dwelling organisms (Borga et al. 2011). Species with a preference for benthic habitats are more likely to have greater exposures to PAH in a polluted environment, than those with a preference for pelagic environments. Whitefish are

occasionally pelagic but mainly feed on benthos (Muir et al. 2010; Scott and Crossman 1979). In contrast burbot are mainly benthic while northern pike prefer shallow, vegetation-rich habitats. Walleye are primarily a littoral zone species but can be found in waters as deep as 20 m (Scott and Crossman 1979). Goldeye occur in turbid slow moving waters of rivers, ponds, and marshes. They are also found in muddy shallow areas of lakes but frequent deeper areas over winter. These trends in species tissue concentrations are also consistent with previously measured concentrations of PAHs, reported as Fluorescently Active Compounds (FACs) in bile (Ohiozebau et al 2015).

Total concentrations of PAHs in fish from Fort McKay and Fort McMurray were significantly greater ($p < 0.01$) than those in fish from Fort Smith and Fort Resolution indicating greater concentrations of PAHs in the AR than in the Slave River (Lanfranchi et al., 2007). Concentrations of PAHs in fishes collected from Fort McMurray can be attributed to natural incision of the river into bitumen, aerial deposition from operations located downstream, operations around Fort McMurray and in the Clearwater River catchment, and finally from general human activity in this increasingly urbanized area.

Many sources may be responsible for the observed PAHs in the collected species. PAHs are generally classified as low molecular weight PAHs (LMW-PAHs; 2- and 3-ring PAHs) compared to larger molecular weight PAHs (HMW-PAHs; 4-6-ring PAHs). The LMW-PAH/HMW-PAH ratios observed in the five species, and seasons from the sampling locations were > 1, indicating mainly petrogenic sources (Rocher et al., 2004). 2- and 3-ring PAHs dominated the distribution at all sampling sites, species and seasons, and accounted for 19.4% and 36.2% of ∑PAHs, respectively (Fig 3.6). Naphthalene was the compound accumulated to the greatest concentration possibly due to its lesser affinity for particles and greater water solubility.

Phenanthrene is a principal PAH component, and was the second most prevalent compound (∑178.8 ng/g) in this study. This is a similar profile of PAH compounds to that generated by petrogenic pollution (Al-Yakoob et al., 1994). Chrysene is normally produced through combustion and was present at a mean concentration of 1.8 ng/g wm. 4-ring PAHs accounted for 24.2% of ∑PAHs. The potentially carcinogenic 5- and 6-ring PAHs were lesser in concentration, accounting for only 15.4% and 4.8% of ∑PAHs, respectively. This result is similar to previous fish studies from similar areas in other parts of the world (Ramalhosa et al., 2012; Nkpaa et al., 2013).

Diet is a major route of human exposure to PAHs (Cheung et al., 2007). In this study the estimated exposure to PAHs through fish consumption does not represent a significant additional risk to human consumers. The foregoing risk assessment does not assess other food sources nor other non-dietary routes to PAH exposure but addresses only additional risk associated with fish consumption. Furthermore, intake of contaminants such as PAHs, should not be the only criterion for consideration when assessing the potential risk to human health-exposure, time and intensity of exposure should also be considered (Binelli and Provini, 2004) as determined in section 3.3.1. Therefore, it is unlikely that PAHs derived from fish consumption in the Athabasca/Slave Rivers would be causing adverse level acute or intermediate effects in humans.

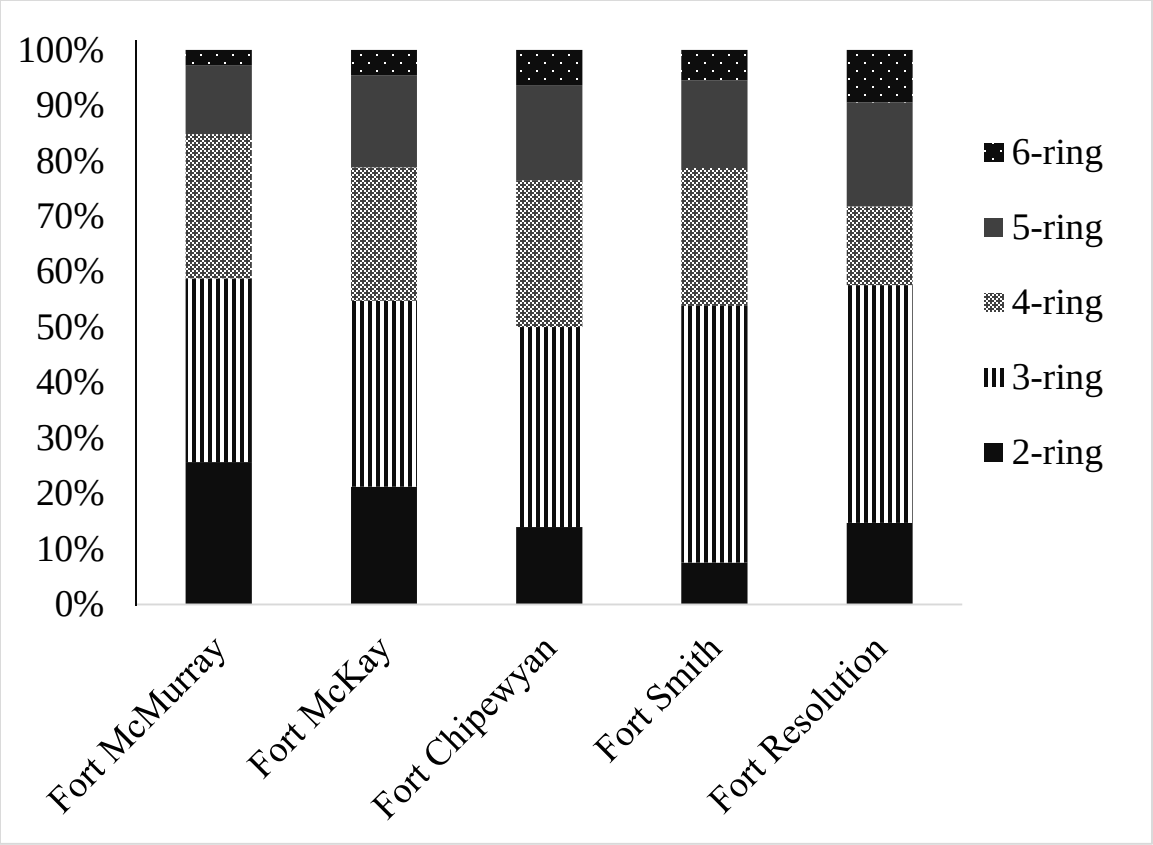


Figure 3.6: Percentage distributions of 2-, 3-, 4-, 5-, 6-ring PAHs in the muscle tissues of whitefish, goldeye, burbot, walleye, and jackfish from the Athabasca/Slave Rivers.

Average values for LCRs for all population groups were less than one in a million chances of additional human cancer over a 70-year lifetime (ILCR = 10^{-6}). From this result, it seems unlikely that PAHs derived from fish collected from the locations in the Athabasca/Slave Rivers would be causing adverse effects in First Nations communities in the areas. However, an individual can be exposed daily to a wide range of contaminants through dietary exposure (Pompa et al., 2003; Wei et al., 2011). Contaminants like heavy metals, PAHs, and naphthenic acids have been reported in air, land, and AR (Kelly et al., 2009 & 2010; Ross et al., 2012; Zhang et al., 2016). Cumulatively, the additive effects may make the LCR values of PAH in fish more significant even if it is less than 1.0, and more than 1 in a million, respectively. The

cumulative and possible interactive effects of these different contaminant groups also need to be considered when assessing risk.

It is difficult to absolutely assess the carcinogenic risk of PAHs because of the inherent uncertainties in risk assessment. For example, different cooking methods could affect different concentration of PAHs in cooked fish (Wretling et al., 2010). Also, possible synergistic and/or antagonistic effect might occur among the observed PAHs that might not have been accounted for during risk assessment. The B[a]P_{eq} based approach does not account for the toxicity of all PAHs, e.g., alkylated compounds, to which the population of interest may be exposed. Also, concentrations of B[a]P_{eq} used in this study to estimate risk were extrapolations from animal toxicity studies, although this risk assessment followed best practice and these values are recommended by the US-EPA and Health Canada, nevertheless, they may not totally reflect the carcinogenic potential of these compounds in humans. Despite its inherent challenges, risk assessment provides a useful framework to evaluate the potential effects of environmental contaminants to humans. In the Athabasca/Slave Rivers, health risk assessment of pollutants, especially from the rapid economic development, is necessary to monitor human and ecological impact in the area. This study, which evaluated the carcinogenic risk level for different population groups in Fort McMurray, Fort McKay, Fort Chipewyan, Fort Smith and Fort Resolution, was an essential first step for a long-term risk assessment in the area. While there are some uncertainties, the overall conservative approach we have taken indicates that there is *de minimis* risk to people from PAHs in fishes that they might consume and thus the fish are safe to eat and to do otherwise would deprive individuals of the positive health benefits on neurobehavioral development and prevention of cardiovascular disease of eating fish.

3.5 Conclusion

This study analyzed 16 PAHs in edible parts of selected fish species in the Athabasca and Slave Rivers. Measurable concentrations of PAHs were detected across spatial and seasonal studies. The profile was dominated by 2-3-ring PAHs, and 4-ring PAHs were also abundant. The spatial distribution of PAHs varied significantly at different sampling locations with the highest concentration in fishes from Fort McKay. Seasonal variations were also observed. Concentrations of ∑PAHs were greater in whitefish than in other species.

Contamination with PAHs detected in the various fishes of the Athabasca/Slave Rivers is likely not a health risk to human consumers in the area. Fresh fish from the Athabasca/Slave Rivers are probably a minor dietary source of PAHs. Emphasis should be placed on science based monitoring in the Athabasca/Slave river system as a whole. It is desirable therefore that a monitoring program in water, sediments, and biota be in place and extend to the entire Athabasca/Slave basin to detect the presence of contaminants and mitigate their potential human and ecological effects. It is not the aim of this paper to diminish the concerns that First Nations Communities have expressed about contamination of fish as a valuable economic and cultural resource. While this paper may assuage some concerns relative to immediate and direct health effects it does not diminish concerns relative to the societal and cultural value of these resources.

Preface to Chapter 4: Pattern Recognition of Alkyl-PAHs in Muscles of Fishes from the Athabasca and Slave Rivers, Canada

My third objective was to use patterns of contamination to provide a scientific basis for elucidating the source of polycyclic aromatic hydrocarbons in the Athabasca and Slave Rivers. Alkyl-PAHs are useful for differentiating petrogenic from pyrogenic sources of hydrocarbon compounds. This study analyzed alkyl-PAHs in muscle samples of 425 fishes from the Athabasca and Slave Rivers using gas chromatography/mass spectrometry. Most of the fish analyzed showed measurable levels of alkyl-PAHs in muscle tissue. In this chapter, I applied multivariate analysis and the ratio of low molecular weight (LMW) to high molecular weight (HMW) parent PAHs and alkyl-PAHs as the major discriminant analyses techniques to characterize PAH profiles in the muscle of fishes from the Athabasca and Slave Rivers.

This manuscript is current in the final stage of internal review process under joint authorship with Brett Tendler (University of Saskatchewan), Erin Kelly (Government of the Northwest Territories), John P. Giesy (University of Saskatchewan), and Paul Jones (University of Saskatchewan). I played a leading role in the research design and sample collection, Mr. Tendler assisted with sample collection. I was solely responsible for the analysis of alkylated PAHs and I wrote and revised this manuscript with editorial comments from the other authors. Dr. Kelly was responsible for coordination with communities, the government of NWT and sponsors. Dr. Giesy provided part of the research funding and editorial feedback. Dr. Jones provided a substantial part of my student and research funding. Furthermore, he coordinated the field work, assisted with experimental design, data interpretation and provided editorial feedback.

CHAPTER 4

Pattern Recognition of Alkyl-PAHs in Muscles of Fishes from the Athabasca and Slave Rivers, Canada

Abstract

This study analyzed alkyl-PAHs in muscle samples of 425 fish from the Athabasca and Slave Rivers. Most of the fish analyzed showed measurable levels of alkyl-PAHs in muscle tissues. The mean concentration of ∑alky-PAHs for the fishes sampled was 68 ng/g, wet mass (wm), alkyl-PAH levels ranged from the detection limit to 134 ng/g, wm. The pattern distribution of the measured alkyl-PAHs and their parent compounds were determined by multivariate analysis using principal component and hierarchical cluster analyses. Naphthalene alkylates made the largest contribution to the PAH budget with 47%, followed by fluorenes with 24%. Alkylation of ∑2-ring (naphthalenes), ∑3-ring (fluorenes and phenanthrene/ anthracene), and ∑4-ring (fluoranthenes and chrysenes/ benz(a)anthracenes) PAHs was observed. The distributions of 2- and 3- ring alkyl-PAHs were generally bell shaped, indicative of PAHs of petrogenic origin. The degree of alkylation was most evident in Fort McKay, followed by Fort McMurray. The general presence of naphthalenes and phenanthrenes and the evaluation of molecular ratios (i.e., LMW/HMW alkyl-PAHs) allow us to conclude that the general source of pollution is petrogenic, probably due to increase in oil sand activities around Fort McMurray and Fort McKay.

4.1 Introduction

In recent years, concerns have been raised about the presence of elevated concentrations of polycyclic aromatic hydrocarbons (PAHs) in the AR (AR), and their possible impact on ecological and human health. This is largely due to the toxicity of PAHs to animals and humans. Higher molecular weight PAHs are either classified as or are suspected to be potent mutagens or carcinogens (Angerer et al., 1997; Huang et al., 2012). Some PAHs, including alkylated chrysene and benz[a]anthracene induce dioxin-like responses via activation of aryl hydrocarbon receptor (Eichbaum et al., 2014; Lee et al., 2015; Lin et al., 2015). Alkylated PAHs (alkyl-PAHs) have potential for chronic toxicity, especially to fish (Lin et al., 2015; Lee et al., 2015). Toxicity in fish could be mediated through the interaction of alkyl-PAHs with cytochrome P450 enzymes (Bauder et al., 2005), narcosis (Turcotte et al., 2011), interaction with cardiac receptors (Incardona et al., 2011), and the Ah receptor (Scot et al., 2011). Tricyclic and tetracyclic alkyl-PAHs with one to four alkyl substituents have been reported to cause a syndrome called blue sac disease (BSD) in early life stages of fish (Adams et al., 2014; Bauder et al., 2005).

PAHs in the environment are mainly derived from petrogenic, pyrogenic, and diagenic sources (Huang et al., 2012; Guo et al., 2007). PAHs can be of natural or anthropogenic origin (Morillo et al., 2008). Natural sources include forest fires, bitumen, oil seeps from crude oil deposits, volcanoes and erosion of carbonaceous plant debris (Jiao et al., 2009; Zakaria et al., 2002). Anthropogenic PAHs in the environment are formed either by incomplete combustion or by thermal alteration of organic matter (Luo et al., 2008). The major sources of PAHs in the Athabasca and Slave Rivers are oil sands deposits and development (Akre et al., 2004; Kelly et al., 2009). As a result, the PAH concentrations in fish muscle and bile have been reported to increase at locations near the Athabaca oil sand formation and operations (Ohiozebau et al., 2015

& 2016). PAH concentrations that exceed Canadian Federal and Provincial guidelines for the protection of aquatic life have been reported in environmental samples close to oil sands operation facilities (Timoney, 2007). PAHs emitted from oil sands operations are mainly transported into river sediments by dry or wet atmospheric deposition, and riverine inflows (Kelly et al., 2009).

PAHs released into the environment are subject to a range of degradation processes, including microbial degradation, evaporation, and photo-oxidation (Baird et al., 2007; Sauer et al., 1998). These weathering processes cause significant changes in the physicochemical profile of PAHs (Yim et al., 2011). During stage I weathering, evaporation is the dominant weathering process and it is characterized by loss of 2-ringed PAHs. The alkyl PAHs of phenanthrene could exhibit loss during stage II weathering. In general, alkyl-PAHs are more resistant to weathering than their parent homologues (Headley et al., 2001). Oil sands deposits have been undergoing weathering over geological time prior to being deposited in the Athabasca and Slave Rivers (Akre et al., 2004).

It is often difficult to define the sources of PAHs in the aquatic environment because of the composite nature of possible sources (Farrington et al., 1977). The source of PAHs in the Athabasca and Slave Rivers, for example, is complex. The Alberta oil sands deposits underlie the AR and tributaries (Conly et al., 2007) and, portions of the AR and its tributaries have naturally incised into the oil sands deposits, releasing hydrocarbon related contaminants, including PAHs, into ambient sediment and biota. In addition to the natural input, oil and gas developments are located near Fort McMurray. Forest fires, agriculture, transport, and population, all of which are expanding, increase the hydrocarbon load of the river, thereby compounding natural and anthropogenic sources. However, it has been reported that the distribution of PAHs varies among

different sources. It is possible to use PAH pattern recognition to link contaminants with their source(s). This strategy uses knowledge of chemistry and fate of individual PAH compounds to characterizing and identify possible sources (Peters et al., 2005). Several applicable and robust techniques are widely used in source identification and allocation of hydrocarbons in mixtures. The major approaches used in source identification include, principal component analysis, and source-specific diagnostic ratios of PAHs and their alkylated homologues (Johnson et al., 2007; Luo et al., 2008; Stout and Graan, 2010).

This study applied multivariate analysis and the ratio of low molecular weight (LMW) to high molecular weight (HMW) parent PAHs and alkyl-PAHs as the major discriminant analyses techniques to characterize PAH profiles in the muscle of fishes from the Athabasca and Slave Rivers. Alkyl-PAHs are useful for differentiating petrogenic from pyrogenic sources of hydrocarbon compounds. Alkyl-PAHs have been used for source identification in the Fraser River (Yunker et al., 2002), Mackenzie delta (Headley et al., 2002), and in tributary sediments of the AR (Evans et al., 2002; Headley et al., 2001).

Baseline studies have been carried out to characterize the natural source of contributions of oil sand materials entering the AR system by studying the concentration of PAHs and their alkylated homologues in sediment samples from the AR (Conly et al., 2002; Headley et al., 2002). Despite the widespread use of PAHs for pattern recognition, most studies have been restricted to specific sample types, e.g., lake or river sediments (Bourbonniere et al. 1995; Evans et al., 2002; Kurek et al., 2013). There are no published data, which comprehensively address the relative origin of PAHs in muscle samples of fishes from locations in the Athabasca and Slave Rivers. We analyzed alkyl-PAHs in muscle of five fish species (425 samples in total) from three

locations in the AR and two locations in the SR. Our findings provide baseline data for future monitoring of alkyl-PAH exposure in the area.

4.2 Materials and Method

4.2.1 Analytical Chemistry Methods

The analytical methods used for extraction are described by Ohiozebau et al., (2015b). In summary, about 15 g wet mass of the samples were homogenized in a glass mortar, dried with sodium sulfate, extracted for 18 h using a soxhlet apparatus with 250 ml dichloromethane (DCM). Extracts were filtered through a glass wool and passed through a column containing silica, acid-silica, and basic-silica for cleanup. The extract was concentrated to 1 ml by rotary vacuum evaporation and spiked with appropriate surrogates (acenaphthyene-d_8, p-terphenyl-d_{14}, benzo[e]pyrene-d_{12}) before further concentration to 0.1 ml under a gentle stream of nitrogen.

The PAH analysis was performed using an Agilent 7890A gas chromatograph interfaced to an Agilent 5975 series Mass Selective Detector and a 7683 series autosampler. Mass spectrometry was initially operated in full-scan mode to determine the retention times of the alkyl-PAHs in a diesel oil before operating in Selected Ion Mode (SIM) for the samples. A high-resolution capillary column was used (J&W fused-silica DB5 column), 60 m long, 025-mm i.d., and 0.25-µm film thickness. The injection temperature was set to 250^0C and the detector to 280^0C. The column was held at 60^0C for 2 min and then ramped at 20^0C/min to 160^0C followed by 5^0C/min to 268^0C and 2^0C/min to 300^0C, where it was held for 10 min to give a total run of 55.5 min. The instrumental limit of detection (LOD) ranged from 0.1- 3.0 ng/g, wet mass (wm). All analytical values less than the quantification limit were replaced with their respective LOD values for statistical analysis.

Mass labelled PAH standards were obtained from Wellington Laboratories (Guelph, Canada). Diesel Oil was purchased from Accustandard Laboratory, Canada. Twenty-eight target analytes included the parent and C-1, C-2, C-3, and C-4 homologues of naphthalene, fluorene, phenenthrene/anthracene, fluoranthene/pyrene, and chresene/benzo(a)anthracene. The nomenclature of PAHs, their abbreviations and retention times are provided (Table 4.1).

Parent PAHs were quantified using internal and recovery standards. Approximate alkyl-PAHs concentrations were calculated using the same response factor for the parent and corresponding alkylated homologue, while identification was based on (a) relative retention time of the target parent PAHs (Fig. 4.1a), (b) comparison with retention times of alkyl-PAHs in diesel oil (Fig. 4.1b), and (c) comparison with reference mass spectra. Data quality objectives included matrix spike recovery between 40 and 120 % for 16 EPA priority PAHs. Surrogate extraction, matrix spike, duplicate analyses and laboratory blanks were analyzed alongside the sample runs to ensure quality control and quality assurance.

Table 4.1: List of target analytes, abrrevations, ions and retention time before detection

PAH Compounds	Abbrevation	Primary Ion (m/z)	Retention Time (min.)
Naphthalene	N_0	128	11.2
C1-Naphthalene	N_1	142	11.5 – 12.8
C2-Naphthalene	N_2	156	13.1 – 14.2
C3-Naphthalene	N_3	170	14.3 – 15.9
C4-Naphthalene	N_4	184	16.0 – 17.4
Fluorene	F_0	166	17.5
C1-Fluorene	F_1	180	17.6 – 18.1
C2-Fluorene	F_2	194	19.1 – 20.9
C3-Fluorene	F_3	208	20.5 – 25.9
C4-Fluorene	F_4	222	21.8 – 27.6

Phenanthrene/Anthracene	P/An	178	22.4
C1-Phenanthrenes/Anthracenes	P_1	192	22.1 – 23.7
C2-Phenanthrenes/Anthracenes	P_2	206	23.8 – 28.9
C3-Phenanthrenes/Anthracenes	P_3	220	24.5 – 30.1
C4-Phenanthrenes/Anthracenes	P_4	234	30.4 – 36.1
Fluoranthene	Fl_0	202	29.8
Pyrene	Py	202	30.5
C1-Fluoranthene/Pyrene	Fl_1	216	31.2 – 33.5
C2-Flyoranthene/Pyrene	Fl_2	230	31.7 – 38.5
C3-Fluoranthene/Pyrene	Fl_3	244	32.9 – 42.3
C4-Fluoranthene/Pyrene	Fl_4	258	39.2 – 45.6
Chrysene	C_0	228	40.2
Benzo(A)Anthracene	Baa	228	40.5
C1-Chrysene/Benzo[A]Anthracene	C_1	242	40.5-42.3
C2-Chrysene/Benzo[A]Anthracene	C_2	256	41.3 – 44.5
C3-Chrysene/Benzo[A]Anthracene	C_3	270	42.3 – 46.3
C4-Chrysene/Benzo[A]Anthracene	C_4	284	45.0 – 50.0

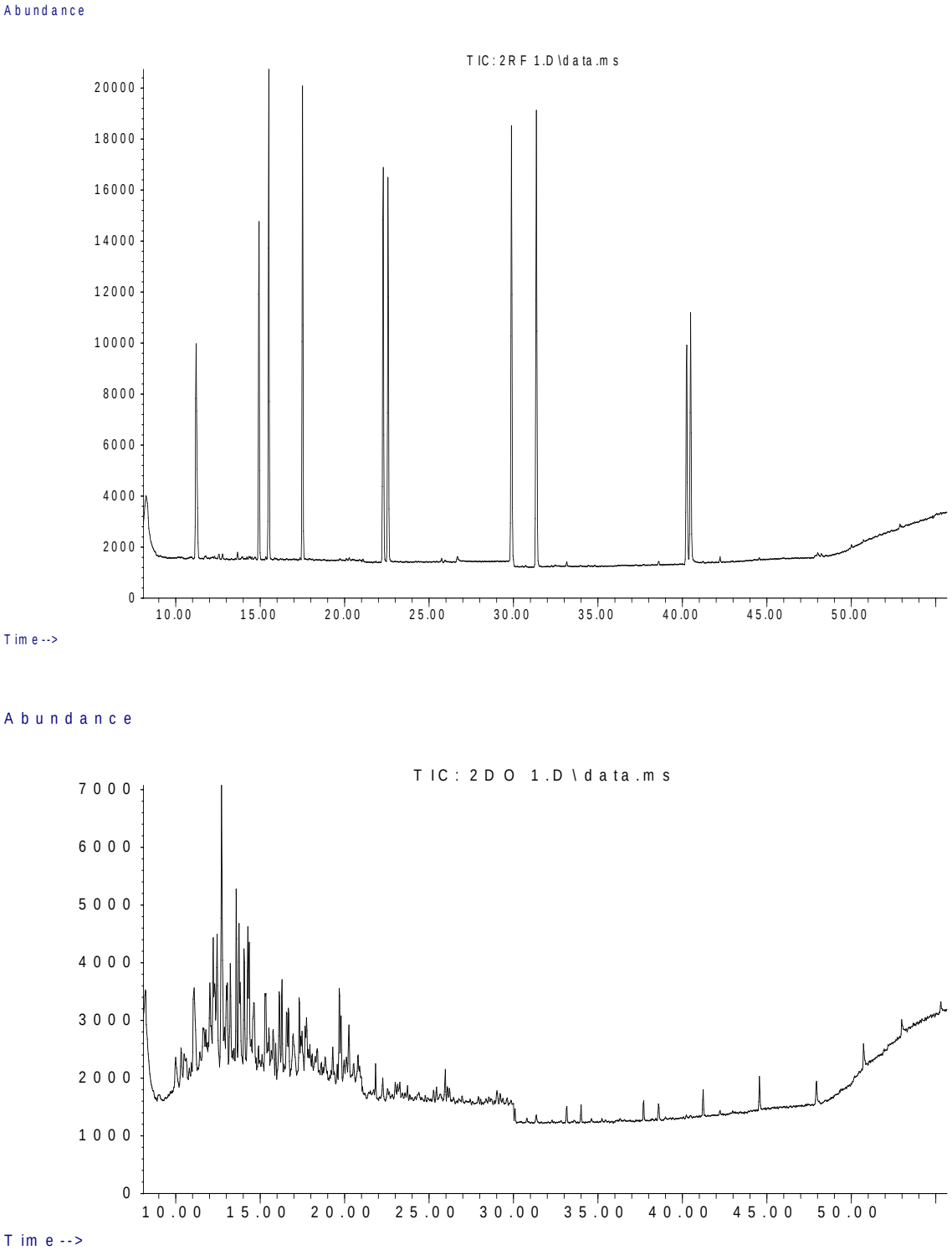


Figure 4.1. a) Typical parent PAH chromatogram using PAH standard showing primary PAH ions (Table 4.1). b)Typical alkylated PAH chromatogram with the respective parent compounds in diesel oil. Traces were obtained using selected ion monitoring mode.

4.2.2 Statistical Analyses

Reported concentrations with respect to wet mass of fish are indicated by the abbreviation wm. The sum of all measured alkyl-PAHs are referred to as ∑alkyl-PAHs. Non-detected concentrations were replaced with their respective LODs. Nonparametric Kruskal-Wallis test was used to test for differences between the fish species, locations, and seasons because normality of the distributions of the residuals were violated. Cluster analysis was conducted by use of 'complete' linkage and with Pearson product-moment correlation coefficient (r) as the distance metric to measure the similarity in patterns among profiles of relative concentrations in the samples. All statistical analyses were conducted with Microsoft Excel, SigmaPlot for Windows, version 11.0 or Systat for Windows, version 12.0. Significance was set at $p < 0.05$.

4.2.3 Multivariate Analyses

The data used to run PCA were untransformed alkyl-PAH concentrations. PCA calculates a "score" for each sample and for each principal component. I further used hierarchical cluster analysis (HCA) to investigate the presence of congener groupings within the data. The Pearson correlation measure used in this study is not influenced by differences in scales between objects. As a result, data scaling, normalization or standardization was not required.

4.3 Results

4.3.1 Contributions from Alkyl-PAHs

Results of the analysis of alkyl-PAHs determined using GC/MS are summarized in Table 4.2. Alkyl-PAHs were detected in all samples of fish muscle collected from the Athabasca and Slave rivers at each location and during each season. The average concentration of ∑alkyl-PAHs in the muscle of the 425 individual fish was 68 ng/g wet mass (wm). Alkylated PAH concentrations ranged from the LOD to 134 ng/g, wm. Naphthalenes had the greatest contribution with 47% to the alky-PAH budget, followed by fluorenes with 24% of the monitored alkylated compounds. The degree of alkylation across the sampled locations are reported (Fig. 4.2). Alkylation for all species, locations, and seasons of ∑2-ring (Naphthalenes), ∑3-ring (Fluorenes and Phenanthrene/ Anthracene), and ∑4-ring (Fluoranthenes and Chrysenes/Benz(a)anthracenes) PAHs are included in Fig. 4.3. The greatest concentration of naphthalenes is at N_2 (Ion 156; 13 ng/g, wm). Similarly, the dialkylated fluorenes and fluoranthene/pyrenes were the most abundant of their respective homologue groups, respectively. The P_3 phenanthrene/anthracene had the greatest concentration while chrysene has the greatest concentration at P_4 (ion 284; 2.3 ng/g, wm). In the summer sampling, an average concentration of 55 ng/g, wm total alkylated PAH was measured but this was considerably greater during the spring sampling season (103 ng/g, wm).

Table 4.2: Mean concentrations of alkylated PAHs from five locations of the Athabasca and Slave Rivers in a) summer 2011, b) fall 2011 and c) spring 2012. n.d = not detected, n.a = not available. All values in ng/g wet mass (wm).

Table 4.2a: 2011 Summer Result

Species	Site	N1	N2	N3	N4	F1	F2	F3	F4	P1	P2	P3	P4	FL 1	FL 2	FL 3	FL 4	C1	C2	C3	C4
Burbot	FR	n.d	n.d	2.6 ±2.0	n.d	n.d	n.d	n.d	1.6 ±2.8	0.9 ±2.4	2.5 ±6.7	0.9 ±1.6	1.2 ±3.6	0.6 ±1.7	3.2 ±9.9	0.8 ±2.2	0.2 ±0.3	0.7 ±1.9	0.8 ±2.2	1.1 ±2.8	0.7 ±0.9
	FS	n.d	n.d	n.d	n.d	2.8 ±2.6	4.8 ±4.6	n.d	3.6 ±3.5	1.0 ±1.2	0.5 ±0.7	0.7 ±0.6	n.d	0.2 ±0.1	0.2 ±0.3	n.d	0.6 ±0.5	n.d	n.d	0.2 ±0.1	0.4 ±0.3
	FC	6.3 ±0.6	8.0 ±1.4	9.5 ±1.0	9.2 ±1.5	2.6 ±2.6	3.5 ±3.6	7.1 ±3.0	n.d	1.1 ±0.9	1.1 ±1.3	2.5 ±3.4	0.6 ±0.5	0.9 ±0.8	4.1 ±5.5	1.6 ±1.6	0.8 ±0.1	0.6 ±0.6	2.4 ±2.8	0.5 ±0.0	0.2 ±0.1
	FM	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a
	FMU	13 ±8.4	19 ±8.5	10 ±3.1	3.9 ±2.4	3.8 ±4.1	8.0 ±8.9	0.6 ±0.5	7.4 ±5.5	2.5 ±2.0	1.6 ±1.9	2.1 ±3.1	3.6 ±5.9	0.2 ±0.1	0.7 ±0.8	0.7 ±0.8	0.8 ±0.9	0.9 ±0.9	0.4 ±0.3	1.2 ±1.9	0.8 ±1.3
Goldeye	FR	13 ±5.6	18 ±11	18 ±8.0	8.8 ±4.1	1.7 ±0.0	3.5 ±1.4	5.8 ±6.2	3.0 ±1.6	n.d	0.3 ±0.0	0.7 ±0.0	0.6 ±0.1	3.9 ±4.5	0.3 ±0.1	0.7 ±0.1	1.8 ±0.4	0.3 ±0.2	0.7 ±0.1	0.2 ±0.2	0.1 ±0.0
	FS	n.d	n.d	5.0 ±5.7	n.d	n.d	2.2 ±2.1	n.d	n.d	n.d	n.d	1.2 ±3.3	n.d	0.5 ±0.7	n.d	n.d	0.5 ±1.0	n.d	n.d	0.2 ±0.2	0.2 ±0.3

Jackfish	FC	1.6 ±1.2	4.7 ±2.9	5.3 ±2.6	n.d	5.4 ±10	n.d	9.6 ±8.6	8.4 ±11	1.8 ±1.3	19 ±15	8.6 ±9.4	10 ±9.3	3.0 ±3.1	11 ±10	6.3 ±6.8	3.4 ±3.2	6.3 ±7.7	8.7 ±8.5	5.1 ±11	4.2 ±7.6
	FM	2.7 ±2.9	8.7 ±7.6	7.9 ±5.4	1.8 ±2.2	3.4 ±4.9	4.0 ±7.0	6.2 ±5.4	5.4 ±10	8.5 ±19	4.1 ±5.2	3.3 ±6.1	2.7 ±4.3	1.0 ±1.7	8.8 ±13	6.0 ±16	3.1 ±6.5	2.8 ±5.5	5.7 ±9.1	1.3 ±.4	2.1 ±2.1
	FM U	3.6 ±3.6	7.3 ±5.2	8.6 ±6.2	2.7 ±2.6	3.1 ±5.8	1.9 ±1.3	9.2 ±10	n.d	1.5 ±2.5	0.3 ±0.4	1.0 ±1.5	0.5 ±0.8	4.9 ±7.2	4.1 ±7.5	3.6 ±7.5	4.5 ±9.0	3.4 ±7.5	2.7 ±4.4	0.5 ±0.6	0.3 ±0.3
	FR	n.d	3.8 ±1.4	3.9 ±4.9	n.d	1.1 ±2.2	n.d	n.d	n.d	n.d	n.d	0.2 ±0.3	n.d	n.d	n.d	n.d	n.d	n.d	n.d	0.1 ±0.2	n.d
	FS	1.6 ±1.6	4.2 ±5.3	4.3 ±4.3	n.d	n.d	n.d	0.7 ±1.3	n.d	n. d	n.d	0.3 ±0.2	0.4 ±1.1	0.2 ±0.2	0.3 ±0.5	0.3 ±0.7	0.3 ±0.7	0.3 ±0.9	0.3 ±0.5	0.2 ±0.6	0.2 ±0.4
	FC	2.7 ±1.9	7.5 ±6.7	8.6 ±6.8	2.7 ±2.0	2.2 ±1.8	3.6 ±2.6	1.0 ±1.5	2.7 ±2.4	0.7 ±0.5	n.d	0.8 ±0.6	0.5 ±1.3	0.1 ±0.1	0.3 ±0.7	0.4 ±0.9	0.5 ±0.8	0.5 ±1.1	0.4 ±0.6	0.4 ±0.6	0.5 ±0.4
	FM	3.8 ±4.2	14 ±11	4.8 ±3.7	6.1 ±5.0	12 ±12	18 ±15	9.0 ±9.7	1.9 ±2.0	1.0 ±1.0	1.4 ±1.7	0.3 ±1.7	0.3 ±0.4	0.7 ±0.6	0.5 ±0.6	0.3 ±0.3	1.2 ±1.4	0.2 ±0.2	0.8 ±1.1	0.9 ±0.7	1.2 ±.5
	FM U	6.6 ±4.2	15 ±9.4	25 ±9.6	10 ±8.0	4.4 ±3.3	9.0 ±11	11 ±12	5.6 ±4.7	1.1 ±1.0	0.4 ±0.5	0.7 ±0.7	0.8 ±1.4	0.7 ±0.7	0.5 ±0.4	0.4 ±0.6	4.5 ±7.0	0.1 ±0.1	0.6 ±1.0	0.7 ±0.7	1.4 ±2.2
Walley e	FR	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a
	FS	1.0 ±0.8	3.7 ±3.3	6.0 ±3.3	1.4 ±2.2	1.1 ±1.0	2.0 ±1.9	0.2 ±0.1	1.5 ±1.4	0.3 ±0.4	0.1 ±0.1	0.5 ±1.0	n.d	0.1 ±0.1	0.1 ±0.1	n.d	0.7 ±1.5	n.d	0.2 ±0.2	0.1 ±0.1	0.3 ±0.2
	FC	3.0 ±1.9	8.7 ±6.0	15 ±6.0	2.9 ±2.2	2.4 ±1.3	3.1 ±2.7	1.4 ±1.0	2.6 ±2.2	0.5 ±0.4	0.3 ±0.1	2.1 ±3.8	0.3 ±0.4	0.4 ±0.3	0.2 ±0.2	0.2 ±0.2	1.1 ±0.5	0.1 ±0.1	0.3 ±0.2	0.4 ±0.2	0.5 ±0.4

White fish	FM	5.6 ±4.4	15 ±12	21 ±12	6.8 ±6.9	3.7 ±4.0	7.3 ±7.6	4.9 ±5.3	6.6 ±6.5	1.0 ±1.2	1.8 ±2.6	3.1 ±4.1	1.2 ±1.2	0.7 ±0.5	1.9 ±3.0	0.7 ±1.0	2.0 ±2.2	0.5 ±0.8	2.5 ±4.7	1.7 ±2.2	1.3 ±2.2
	FMU	4.4 ±2.9	10 ±5.4	18 ±8.5	6.3 ±9.1	4.1 ±3.9	7.5 ±8.0	5.4 ±8.5	5.3 ±5.8	1.5 ±1.3	1.0 ±1.1	2.2 ±2.7	1.0 ±1.5	0.4 ±0.3	0.9 ±1.3	0.5 ±0.4	1.2 ±0.9	0.2 ±0.2	0.9 ±0.8	0.8 ±0.9	1.1 ±1.0
	FR	1.4 ±1.6	4.2 ±4.3	3.6 ±2.1	1.5 ±1.6	0.7 ±0.7	1.1 ±0.9	n.d	2.0 ±1.8	0.3 ±0.3	0.7 ±1.3	0.4 ±0.6	0.3 ±0.6	0.7 ±1.1	0.4 ±0.6	0.2 ±0.2	0.2 ±0.1	0.2 ±0.2	0.3 ±0.5	0.8 ±1.6	0.9 ±1.2
	FS	1.5 ±1.1	7.1 ±4.3	6.2 ±2.8	2.0 ±1.9	1.1 ±0.7	2.1 ±1.6	0.7 ±0.4	1.4 ±0.9	0.3 ±0.2	0.1 ±0.1	0.2 ±0.1	0.2 ±0.3	0.2 ±0.1	n.d	n.d	0.3 ±0.2	n.d	0.2 ±0.1	0.1 ±0.1	0.1 ±0.0
	FC	2.9 ±1.0	8.4 ±3.6	12 ±5.7	4.4 ±2.3	0.7 ±0.5	1.3 ±0.6	1.8 ±0.8	0.9 ±0.8	0.3 ±0.1	2.9 ±5.3	0.8 ±1.1	1.0 ±1.1	3.2 ±6.8	3.8 ±8.2	1.9 ±4.1	0.3 ±0.2	3.2 ±7.1	0.2 ±0.1	0.1 ±0.1	2.5 ±4.6
	FM	4.3 ±2.9	14 ±5.1	16 ±4.3	2.5 ±1.3	1.0 ±1.2	1.9 ±2.7	3.8 ±4.1	4.7 ±8.9	1.0 ±2.0	1.5 ±2.6	0.9 ±1.2	1.2 ±1.3	4.1 ±8.7	1.8 ±3.0	0.6 ±0.7	0.4 ±0.4	0.4 ±0.5	0.6 ±0.7	0.7 ±1.1	0.3 ±0.4
	FMU	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a

Table 4.2 b: 2011 Fall Result

Species	Site	N1	N2	N3	N4	F1	F2	F3	F4	P1	P2	P3	P4	FL 1	FL 2	FL 3	FL 4	C1	C2	C3	C4
Burbot	FR	1.7 ±1.7	6.2 ±1.6	3.2 ±1.3	1.3 ±1.1	n.d	n.d	n.d	n.d	n.d	n.d	0.3 ±0.2	2.4 ±3.3	0.3 ±0.9	0.4 ±1.0	0.6 ±1.5	n.d	6.0 ±4.7	0.3 ±0.6	0.1 ±0.1	0.1 ±0.1
	FS	3.4 ±1.2	8.6 ±3.5	8.9 ±3.5	2.7 ±0.4	n.d	n.d	n.d	2.4 ±3.1	n.d	0.9 ±0.9	2.7 ±3.7	0.6 ±0.9	0.1 ±0.1	0.1 ±0.2	0.3 ±0.3	n.d	6.7 ±8.9	0.3 ±0.4	1.5 ±2.1	0.1 ±0.1
	FC	6.7 ±0.1	16 ±2.5	11 ±2.4	4.1 ±2.8	n.d	1.8 ±0.9	n.d	2.2 ±2.8	n.d	0.4 ±0.4	1.9 ±2.6	2.6 ±4.0	n.d	1.8 ±2.3	4.0 ±3.5	0.2 ±0.1	1.6 ±2.4	2.2 ±3.7	1.2 ±1.6	0.1 ±0.1
	FM	2.9 ±0.2	6.0 ±0.4	5.4 ±0.0	3.0 ±0.5	1.0 ±0.6	1.8 ±1.8	1.5 ±0.2	1.3 ±1.4	n.d	0.8 ±0.3	1.0 ±0.1	32 ±10	n.d	n.d	5.2 ±3.3	0.3 ±0.4	14 ±9.9	n.d	n.d	0.4 ±0.2
	FMU	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a
Goldeye	FR	1.5 ±1.2	3.8 ±0.8	3.6 ±2.6	1.5 ±1.4	n.d	n.d	2.7 ±5.0	n.d	n.d	n.d	0.4 ±0.3	2.5 ±2.6	1.8 ±3.5	0.1 ±0.2	0.2 ±0.3	0.6 ±1.0	1.6 ±2.9	n.d	0.1 ±0.1	0.1 ±0.1
	FS	1.4 ±0.6	7.6 ±5.6	2.4 ±0.8	1.0 ±0.4	5.3 ±6.8	1.0 ±0.7	1.2 ±0.9	n.d	n.d	1.4 ±2.5	0.2 ±0.2	1.0 ±1.3	2.7 ±4.4	1.1 ±1.3	1.1 ±1.3	0.5 ±0.7	2.8 ±2.7	0.3 ±0.2	4.3 ±4.9	0.2 ±0.1
	FC	4.7 ±1.6	6.6 ±1.6	7.2 ±2.2	5.1 ±2.3	n.d	1.5 ±1.5	6.1 ±2.9	n.d	4.3 ±5.0	0.4 ±0.3	0.8 ±0.7	1.5 ±1.8	1.3 ±0.7	6.0 ±5.1	1.3 ±0.3	3.3 ±2.7	5.2 ±6.9	6.0 ±5.2	1.2 ±1.8	1.2 ±1.5
	FM	5.2 ±4.6	20 ±13	7.2 ±5.8	3.2 ±2.2	10 ±11	5.2 ±5.8	5.0 ±3.4	3.2 ±4.6	1.7 ±2.2	3.6 ±8.0	0.4 ±0.4	0.5 ±0.9	3.8 ±5.0	0.7 ±0.7	3.2 ±4.8	3.0 ±3.9	2.4 ±4.5	1.3 ±1.1	0.6 ±0.5	0.7 ±0.8
	FMU	7.1	12	12	12	1.9	4.4	1.0	3.6	0.8	0.5	1.3	n.d	n.d	0.2	0.3	0.7	0.3	0.3	0.1	0.2
Jackfish	FR	n.d	4.5 ±3.8	3.5 ±3.1	n.d	2.4	n.d	0.6 ±0.6	n.d	n.d	n.d	0.3 ±0.3	n.d	0.4 ±0.7	0.1 ±0.1	0.1 ±0.1	0.1 ±0.1	1.6 ±3.1	0.2 ±0.3	0.1 ±0.1	0.1 ±0.2
	FS	1.1 ±0.7	8.1 ±4.7	4.8 ±6.5	1.3 ±0.8	2.1 ±2.1	1.7 ±2.1	0.6 ±0.7	n.d	n.d	n.d	0.3 ±0.2	n.d	n.d	n.d	n.d	0.3 ±0.4	0.4 ±0.8	0.1 ±0.2	0.1 ±0.1	0.1 ±0.2
	FC	4.2 ±4.2	15 ±7.7	12 ±12	3.9 ±3.0	n.d	1.9 ±1.4	2.4 ±1.8	1.2 ±1.0	0.5 ±0.2	0.5 ±0.5	0.3 ±0.3	2.4 ±4.6	0.9 ±1.4	0.2 ±0.2	1.5 ±3.1	0.2 ±0.2	1.5 ±3.1	0.3 ±0.2	0.3 ±0.3	0.2 ±0.1
	FM	7.8 ±5.5	21 ±11	20 ±17	12 ±10	5.6 ±7.5	14 ±22	5.8 ±8.1	12 ±21	2.0 ±3.2	1.1 ±1.6	1.7 ±2.1	1.3 ±2.7	2.3 ±3.3	1.2 ±1.6	1.8 ±3.3	4.3 ±9.1	3.7 ±6.8	0.8 ±0.7	1.3 ±1.7	1.8 ±3.0

Walleye	FMU	1.0	6.7	3.9	2.7	3.1	7.7	32	2.4	1.9	0.8	0.3	1.5	0.7	0.5	0.6	0.2	0.4	0.5	1.2	0.6
		±0.1	±2.2	±1.4	±2.2	±2.6	±6.9	±44	±0.1	±2.2	±1.0	±0.4	±2.1	±0.8	±0.4	±0.8	±0.2	±0.5	±0.7	±1.3	±0.6
	FR	1.1	10	2.7	0.9	1.6	2.5	2.2	0.6	0.6	1.0	0.3	0.6	0.4	0.2	0.2	0.9	0.3	0.2	0.3	0.5
		±1.0	±6.0	±0.8	±1.6	±1.0	±2.0	±3.9	±0.5	±0.5	±1.9	±0.2	±1.5	±0.3	±0.2	±0.3	±1.8	±0.3	±0.2	±0.6	±1.1
	FS	1.6	5.9	3.8	1.5	1.0	1.9	0.7	1.0	n.d	0.7	0.4	5.1	n.d	0.1	1.2	2.1	2.3	0.2	0.1	0.1
		±0.8	±3.5	±2.2	±0.7	±0.8	±2.3	±0.4	±1.1		±1.0	±0.6	±7.1		±0.1	±2.3	±5.0	±2.9	±0.2	±0.1	±0.1
	FC	1.4	10	2.6	0.8	4.7	5.3	1.1	0.9	1.7	0.4	0.6	n.d	0.5	0.2	0.2	0.2	0.5	0.2	0.2	0.1
		±0.3	±3.5	±1.7	±0.5	±3.5	±2.9	±0.6	±0.9	±1.0	±0.3	±0.5		±0.5	±0.1	±0.2	±0.2	±0.5	±0.2	±0.1	±0.1
	FM	6.3	23	14	5.1	6.7	11	3.6	5.1	2.5	1.0	2.9	n.d	0.6	0.9	0.5	1.1	0.8	1.0	1.1	0.3
		±3.5	±7.1	±10	±3.6	±5.3	±8.1	±8.1	±6.9	±2.1	±0.9	±6.3		±0.4	±0.6	±0.4	±1.7	±0.4	±1.3	±1.6	±0.4
Whitefish	FMU	1.9	8.3	6.9	2.6	0.9	2.3	0.4	1.9	n.d	n.d	0.4	n.d	0.2	0.2	n.d	0.6	n.d	n.d	0.3	0.3
		±0.9	±2.0	±2.2	±0.4	±0.5	±1.4	±0.2	±1.1			±0.4		±0.1	±0.1		±0.6			±0.2	±0.2
	FR	2.3	7.8	7.8	1.6	2.3	2.0	2.4	1.2	0.6	2.0	2.0	0.4	0.9	0.6	0.9	0.5	2.3	0.9	0.7	0.2
		±1.5	±4.5	±5.1	±1.3	±1.3	±2.2	±1.4	±2.0	±0.3	±4.2	±5.1	±0.8	±0.8	±0.9	±1.5	±0.5	±5.5	±1.3	±1.0	±0.3
	FS	2.1	7.9	4.7	1.3	0.6	0.5	0.8	0.3	0.2	0.7	0.8	1.1	n.d	1.6	0.7	n.d	2.1	0.5	0.3	0.4
		±1.3	±5.0	±2.6	±1.3	±0.6	±0.5	±0.5	±0.4	±0.1	±1.1	±1.6	±1.8		±3.9	±1.1		±2.4	±1.1	±0.5	±0.2
	FC	2.1	9.0	9.3	2.0	2.9	2.0	5.5	0.6	2.6	2.6	0.6	9.5	3.7	3.6	7.1	2.0	2.6	10	0.1	10
		±1.2	±5.7	±6.6	±1.9	±3.0	±2.5	±10	±1.2	±6.7	±6.6	±0.9	±15	±6.2	±7.8	±18	±3.6	±6.0	±26	±0.2	±30
	FM	2.6	18	19	3.1	9.9	8.0	4.4	4.1	1.4	0.8	0.8	0.4	0.6	1.2	1.1	1.2	0.5	0.9	0.4	1.1
		±1.5	±8.4	±13	±1.8	±16	±7.5	±4.9	±4.4	±1.2	±1.0	±0.6	±0.8	±0.9	±2.6	±2.1	±2.0	±0.8	±1.2	±0.7	±1.3
	FMU	3.6	20	9.9	2.4	11	6.6	8.7	5.3	2.3	0.8	0.7	n.d	1.3	0.6	0.8	3.4	0.5	0.8	2.7	0.6
		±2.5	±14	±14	±2.2	±12	±3.7	±12	±4.8	±2.2	±0.7	±0.7		±1.7	±0.4	±0.5	±6.6	±0.5	±0.7	±5.2	±0.8

Table 4.2 c: 2012 Spring Result

Species	Site	N1	N2	N3	N4	F1	F2	F3	F4	P1	P2	P3	P4	FL 1	FL 2	FL 3	FL 4	C1	C2	C3	C4
Burbot	FR	1.2 ±0.4	5.8 ±1.2	3.5 ±3.1	1.9 ±0.7	1.2 ±1.0	3.2 ±2.6	0.4 ±0.1	2.3 ±1.8	0.6 ±0.6	0.3 ±0.2	0.8 ±0.9	0.1 ±0.2	0.1 ±0.1	0.1 ±0.1	0.1 ±0.1	0.3 ±0.3	12 ±21	0.3 ±0.1	n.d	0.2 ±0.2
	FS	n.d	6.8	6.0	n.d	1.2	2.5	0.6	2.1	n.d	n.d	n.d	n.d	0.4	0.5	0.9	0.3	1.7	0.3	0.9	0.8
	FC	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a
	FM	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a
	FMU	7.2 ±4.2	39 ±11	24 ±16	5.3 ±5.1	4.1 ±2.2	8.3 ±4.7	2.4 ±2.5	4.2 ±1.9	10 ±0.6	0.7 ±0.3	1.4 ±1.0	0.8 ±1.4	1.1 ±0.9	0.7 ±0.5	0.5 ±0.3	2.3 ±1.8	1.3 ±1.7	0.4 ±0.3	0.6 ±0.7	11 ±19
Goldeye	FR	0.9 ±0.8	7.1 ±3.9	3.5 ±2.0	1.2 ±0.9	3.3 ±9.3	2.4 ±2.3	2.5 ±2.4	1.0 ±1.0	0.6 ±0.8	0.7 ±4.9	0.2 ±0.9	0.2 ±0.7	3.7 ±2.0	0.2 ±3.6	2.0 ±3.3	0.7 ±0.6	0.4 ±0.6	0.4 ±7.3	0.8 ±0.6	0.3 ±0.2
	FS	0.6 ±0.2	2.5 ±1.6	1.9 ±1.1	0.5 ±0.3	1.6 ±1.4	5.0 ±3.4	1.4 ±1.2	5.6 ±5.2	0.5 ±0.3	2.5 ±4.6	0.2 ±0.2	2.4 ±4.3	2.3 ±4.4	1.5 ±2.8	0.9 ±1.6	0.3 ±0.4	0.5 ±1.0	0.3 ±0.3	2.7 ±4.9	0.5 ±0.6
	FC	1.9 ±0.9	7.5 ±1.6	4.7 ±6.0	2.1 ±1.3	1.5 ±0.7	3.9 ±1.3	1.0 ±0.6	2.7 ±1.3	0.7 ±0.4	0.2 ±0.1	1.0 ±0.6	n.d	0.1 ±0.1	1.0 ±0.6	n.d	n.d	n.d	n.d	0.2 ±0.1	0.4 ±0.2
	FM	8.0 ±7.1	30 ±17	31 ±17	11 ±15	7.5 ±8.8	6.6 ±7.2	14 ±10	14 ±24	4.1 ±4.8	8.4 ±12	17 ±31	5.2 ±6.8	7.8 ±6.6	26 ±52	10 ±13	4.1 ±5.4	8.0 ±10	6.4 ±11	4.2 ±3.7	7.4 ±16
	FMU	9.2 ±8.2	47 ±35	14 ±9.9	11 ±11	1.8 ±0.9	3.5 ±2.3	5.0 ±2.9	12 ±13	0.7 ±0.4	6.2 ±11	6.4 ±12	2.6 ±4.0	1.8 ±1.9	11 ±18	2.3 ±3.4	0.6 ±2.0	1.7 ±2.0	2.1 ±3.0	1.9 ±2.5	12 ±15
Jackfish	FR	1.2 ±1.1	5.7 ±2.2	5.0 ±1.8	1.8 ±1.5	0.7 ±0.8	1.3 ±1.4	0.4 ±0.5	1.1 ±1.4	0.2 ±0.3	0.4 ±0.9	0.5 ±0.9	n.d	n.d	0.4 ±1.0	0.6 ±1.6	0.3 ±0.5	1.5 ±4.5	0.2 ±0.5	0.2 ±0.3	0.4 ±0.7
	FS	1.2 ±0.6	3.5 ±1.0	5.4 ±1.7	1.5 ±1.0	1.9 ±2.6	5.2 ±5.5	0.8 ±1.5	3.6 ±4.5	0.9 ±1.5	0.3 ±0.2	1.9 ±2. -	n.d	0.6 ±0.6	0.2 ±0.3	0.1 ±0.1	2.0 ±2.7	0.1 ±0.2	0.7 ±1.0	0.4 ±0.3	0.7 ±0.6
	FC	3.6 ±3.4	9.8 ±4.3	10 ±10	4.4 ±3.9	2.2 ±1.5	3.9 ±2.9	3.5 ±5.2	2.8 ±2.4	0.5 ±0.4	0.9 ±0.8	0.8 ±0.5	0.7 ±0.8	1.3 ±1.9	0.2 ±0.1	0.3 ±0.2	0.4 ±0.4	8.0 ±11	0.7 ±0.7	0.4 ±0.3	0.9 ±1.7
	FM	7.0 ±4.0	49 ±23	43 ±22	6.2 ±2.0	17 ±9.5	38 ±21	9.1 ±3.8	22 ±16	15 ±19	2.5 ±3.3	19 ±24	n.d	5.8 ±8.6	3.9 ±5.5	9.9 ±12	9.4 ±11	3.5 ±2.9	16 ±21	6.1 ±9.1	2.0 ±1.8
	FMU	16 ±7.9	42 ±12	19 ±14	13 ±7.9	10 ±28	4.7 ±5.2	20 ±11	2.6 ±1.8	2.5 ±1.6	1.6 ±1.4	1.6 ±1.0	1.3 ±1.2	2.6 ±2.8	0.7 ±0.5	0.9 ±0.5	2.8 ±2.5	3.5 ±2.3	0.9 ±0.5	3.8 ±3.3	26 ±18

Walley e	FR	1.3 ±0.8	4.3 ±1.6	3.4 ±1.2	1.3 ±1.0	0.9 ±0.6	2.0 ±1.5	0.8 ±0.6	2.3 ±2.3	0.4 ±0.3	1.3 ±3.7	0.4 ±0.3	0.3 ±0.9	0.4 ±0.3	1.4 ±3.0	1.0 ±1.8	0.9 ±1.0	0.9 ±2.1	0.6 ±1.1	0.3 ±0.2	0.1 ±0.1
	FS	2.3 ±1.5	7.7 ±3.2	7.3 ±3.6	2.8 ±2.2	1.9 ±1.7	3.1 ±2.3	1.3 ±1.(	1.0 ±0.6	0.8 ±0.9	0.2 ±0.2	0.7 ±0.5	n.d	0.2 ±0.1	0.1 ±0.1	0.1 ±0.1	0.3 ±0.4	n.d	n.d	n.d	0.1 ±0.2
	FC	3.9 ±1.7	10 ±4.6	12 ±5.6	3.4 ±2.5	1.5 ±1.2	3.8 ±4.3	1.3 ±1.4	2.6 ±3.4	0.4 ±0.3	0.3 ±0.5	0.4 ±0.5	0.3 ±0.3	0.3 ±0.3	0.4 ±0.6	0.2 ±0.3	0.6 ±1.0	0.1 ±0.2	0.4 ±0.5	0.2 ±0.3	0.3 ±0.4
	FM	12 ±10	54 ±30	46 ±33	13 ±8.3	11 ±8.1	19 ±14	23 ±32	11 ±9.7	5.8 ±5.2	4.6 ±3.6	21 ±44	3.1 ±6.9	14 ±13	6.6 ±6.9	3.9 ±4.4	20 ±24	2.2 ±3.2	17 ±24	5.0 ±6.4	11 ±11
	FMU	7.3 ±2.6	27 ±15	14 ±9.0	9.0 ±11	15 ±18	17 ±12	8.5 ±5.6	11 ±11	3.6 ±3.9	1.4 ±0.7	4.2 ±3.6	1.3 ±1.6	1.3 ±0.7	1.3 ±0.7	1.6 ±1.3	1.9 ±1.4	2.9 ±2.0	1.0 ±0.7	2.2 ±2.4	22 ±20
White fish	FR	1.6 ±1.0	5.6 ±2.9	5.2 ±3.2	1.4 ±0.7	0.8 ±0.6	2.1 ±1.6	0.7 ±0.7	1.6 ±1.3	0.7 ±1.2	0.2 ±0.2	0.3 ±0.2	2.7 ±7.1	0.2 ±0.3	0.4 ±1.0	3.5 ±8.5	1.7 ±4.1	2.9 ±7.2	4.1 ±11	0.1 ±0.1	0.3 ±0.4
	FS	1.5 ±0.9	7.0 ±1.1	6.3 ±4.1	2.1 ±1.7	5.4 ±5.4	6.1 ±4.8	4.8 ±2.3	2.8 ±2.4	1.6 ±1.7	0.9 ±0.4	1.0 ±0.8	n.d	0.3 ±0.1	0.3 ±0.1	0.9 ±1.3	1.5 ±1.5	n.d	0.5 ±0.1	0.6 ±0.5	0.5 ±0.7
	FC	1.3 ±0.3	6.7 ±3.4	7.3 ±3.9	2.1 ±1.6	1.2 ±0.6	2.8 ±0.8	0.8 ±0.5	2.1 ±1.2	0.3 ±0.2	0.2 ±0.2	0.7 ±0.9	n.d	0.2 ±0.2	0.1 ±0.1	0.2 ±0.2	0.3 ±0.2	0.1 ±0.1	0.3 ±0.3	0.1 ±0.1	0.4 ±0.2
	FM	12 ±4.3	31 ±6.0	33 ±7.7	7.9 ±4.2	3.4 ±2.2	6.8 ±0.9	5.3 ±3.3	5.1 ±1.3	1.2 ±1.2	0.9 ±0.6	0.3 ±0.3	2.7 ±3.8	2.8 ±1.2	4.9 ±3.7	13 ±13	2.7 ±2.8	12 ±15	1.9 ±2.4	0.6 ±0.6	2.3 ±1.5
	FMU	20 ±22	56 ±56	27 ±29	28 ±44	6.8 ±5.4	13 ±15	4.9 ±3.4	9.3 ±9.4	1.4 ±1.4	2.6 ±2.6	3.1 ±2.2	1.2 ±2.5	3.4 ±3.6	2.0 ±2.8	0.8 ±0.8	2.2 ±2.7	1.9 ±1.1	0.6 ±0.4	2.1 ±2.4	22 ±19

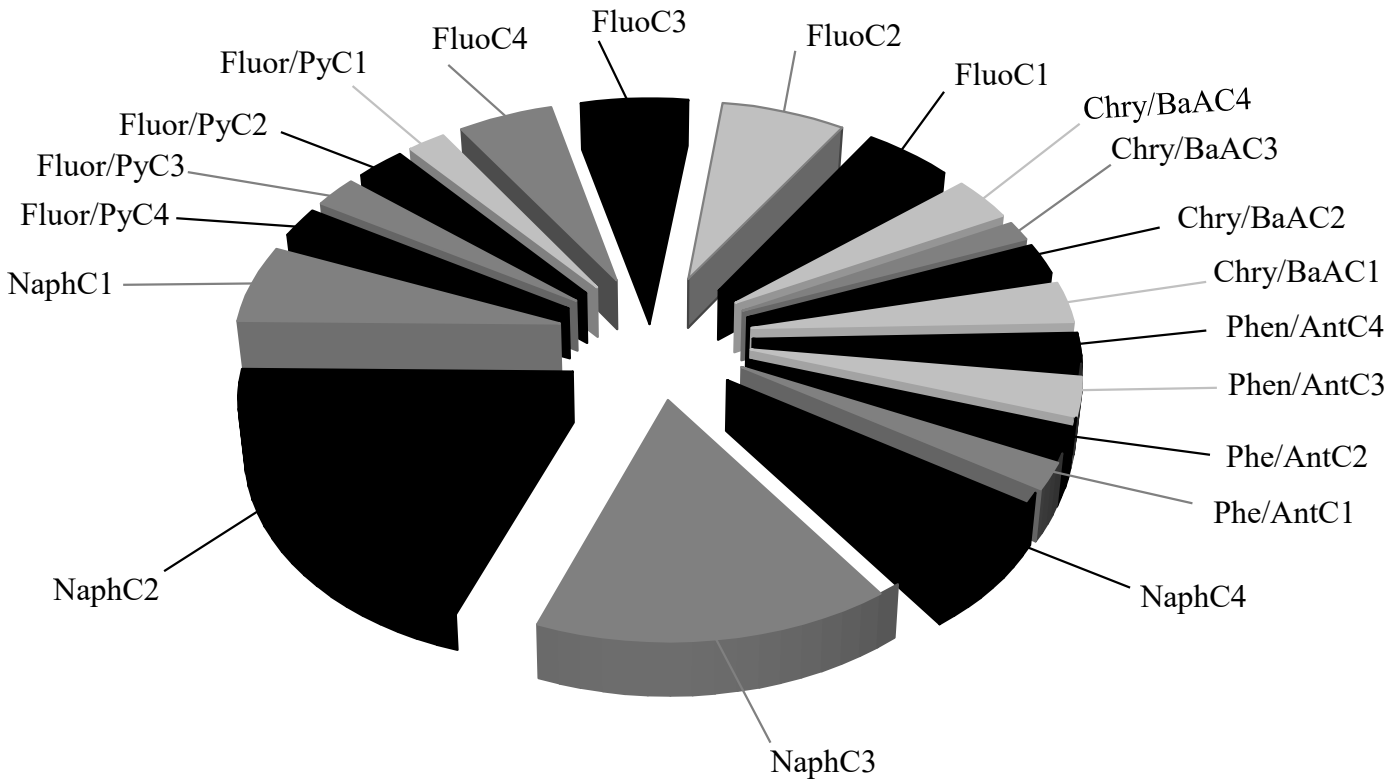


Figure 4.2. The percentage distribution (%) of targeted alkyl-PAHs in fish samples from the Athabasca and Slave Rivers. Naph C1-C4 =alkylated napthalenes; Fluo C1-C4 = alkylated fluorenes; Fluor/Py C1-C4 = alkylated fluoranthenes/pyrenes; Chry/BaA C1-C4 = alkylated chrysenes/Benzo[a]pyrenes; Phe/Ant C1-C4 = alkylated phenanthrenes/anthracenes.

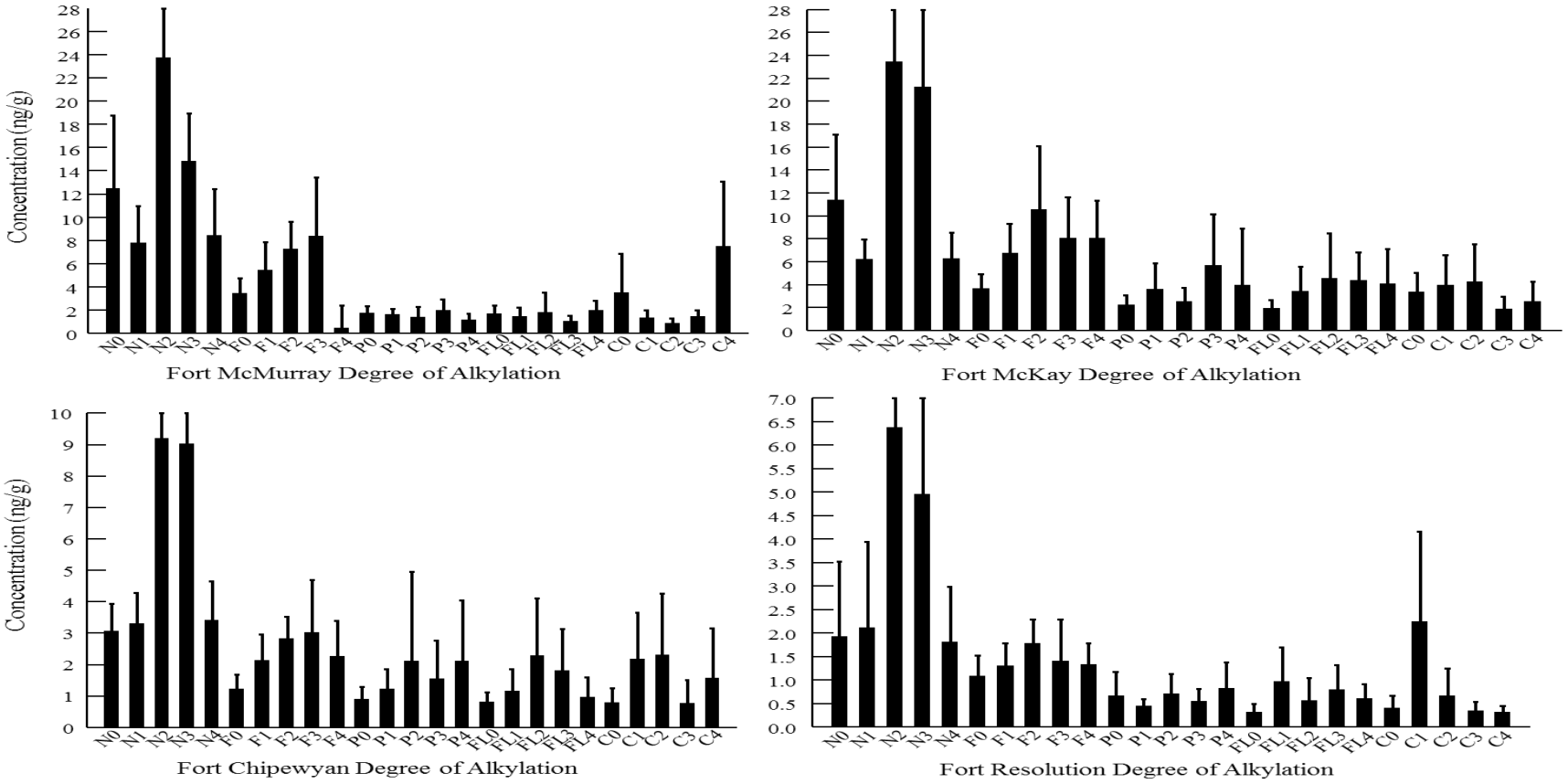


Figure 4.3: Comparison of degree of alkylation from across the locations. X-axis key: N-naphthalenes; F-fluorenes; P-phenanthrenes/anthracenes; FL-fluoranthenes; C-chrysenes showing the distribution of the C_0–C_4 alkylated PAHs: Naphthalene, showing the bell shape, diagnostic of a predominant petrogenic source. Fluorene, showing the bell shape, diagnostic of a predominant petrogenic source. Overall shape and concentration of alkylated analogues are indicative of a predominant petrogenic source.

Table 4.3 shows that significant differences in concentrations of ∑alkyl-PAHs in muscle were observed for goldeye, jackfish, walleye, whitefish and burbot among locations. There was no significant difference by species for seasonal (Table 4.3). In general, greater concentrations of parent PAHs and ∑alkyl-PAH were detected in fishes collected from the AR relative to the SR (Fig. 4.4). Fort McKay had the greatest concentration of ∑alkyl-PAHs, ranging from 61 ng/g, wm (whitefish, summer) to 304 ng/g, wm (walleye, spring) with mean value of 135 ng/g, wm. The concentration of ∑alkyl-PAHs at Fort McMurray ranged from 28 ng/g, wm (walleye, fall) to 208 ng/g, wm (whitefish, spring) with 104 ng/g, wm, mean value. The concentration of Fort Chipewyan ranged from 27 ng/g, wm (whitefish, spring) to 124 ng/g, wm (goldeye, summer) with 55 ng/g, wm, mean value. The fish samples from Fort Smith and Fort Resolution exhibited the least concentration of ∑alkyl-PAHs. The value of Fort Smith ranged from 17 ng/g, wm (goldeye, summer) to 44 ng/g, wm (whitefish, spring) and Fort Resolution varied from 13 ng/g, wm (jackfish, summer) to 82 ng/g, wm (goldeye, summer) with mean values of 28 and 30 ng/g, wm, respectively. There were no significant differences in concentrations of ∑total alkyl-PAHs/total parent PAHs in muscle by species for location and season (Table 4.3).

In general, the contribution of alkyl-PAHs was relatively consistent across locations when the ratio of total alkyl-PAHs to total parent PAHs was compared by species. However, in goldeye and jackfish from Fort Chipewyan, the relative amounts of total alkyl-PAHs were somewhat higher (Fig. 4.5).

Table 4.3: Kruskal-Wallis non-parametric one-way analysis of variance test showing the within species statistic and P values for all sites and seasons using total alky-PAHs and total alkyl-PAHs/total PAHs.

	Total Alkyl-PAHs				Total Alkyl-PAHs/Total PAHs			
	Location		Season		Location		Seasons	
Species	Statistic	p-Value	Statistic	p-Value	Statistic	p-Value	Statistic	p-Value
Walleye	53.88	0.000	2.43	0.297	4.09	0.394	6.91	0.032
Goldeye	32.61	0.000	3.44	0.179	12.79	0.012	6.82	0.033
Jackfish	56.05	0.000	3.41	0.182	2.11	0.716	0.56	0.755
Burbot	21.37	0.000	5.90	0.052	0.83	0.935	14.39	0.001
Whitefish	45.56	0.000	8.15	0.017	10.07	0.039	2.15	0.341

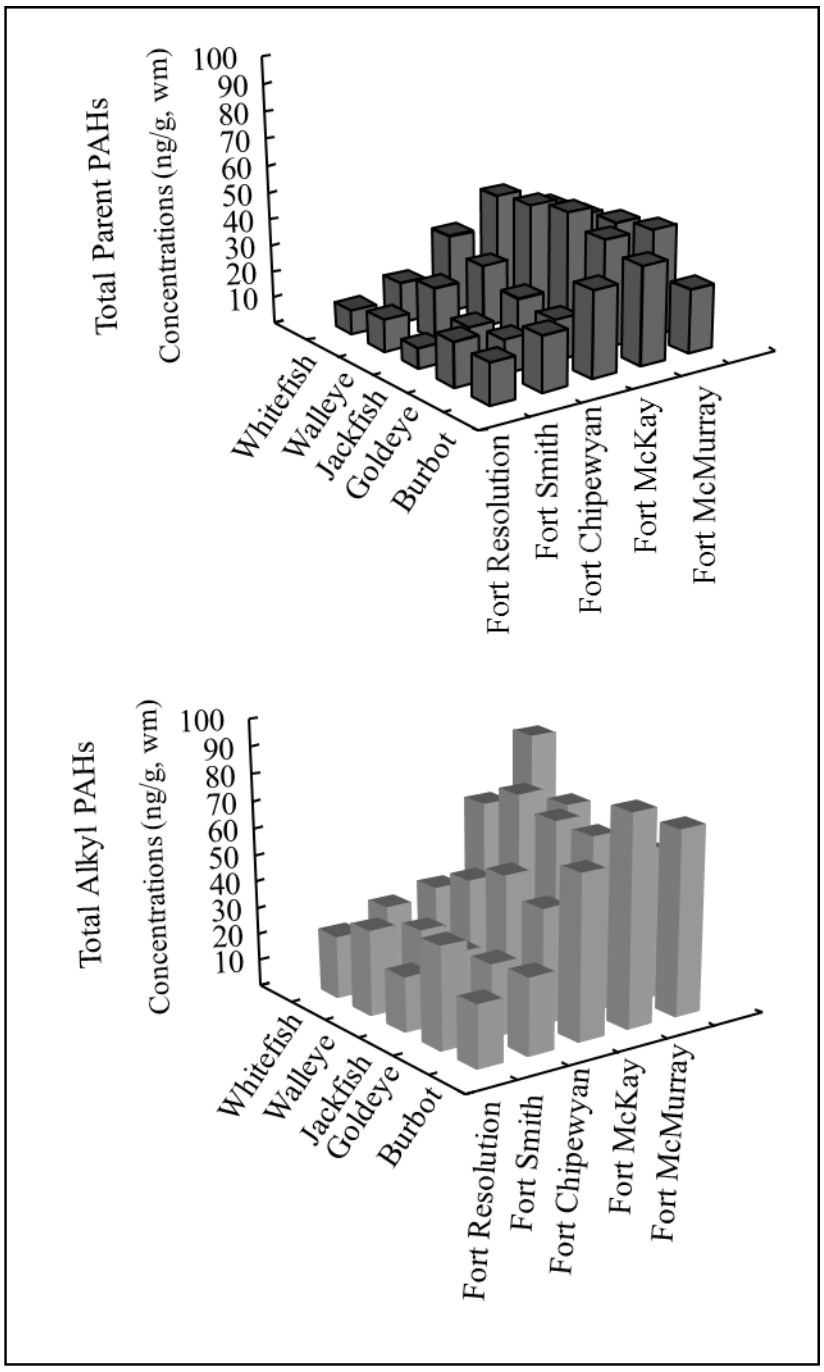


Figure 4.4: Concentrations of total parent PAHs (ng/g, wm) and total alkyl-PAHs (ng/g, wm) in fish muscle by species from the SR and ARs.

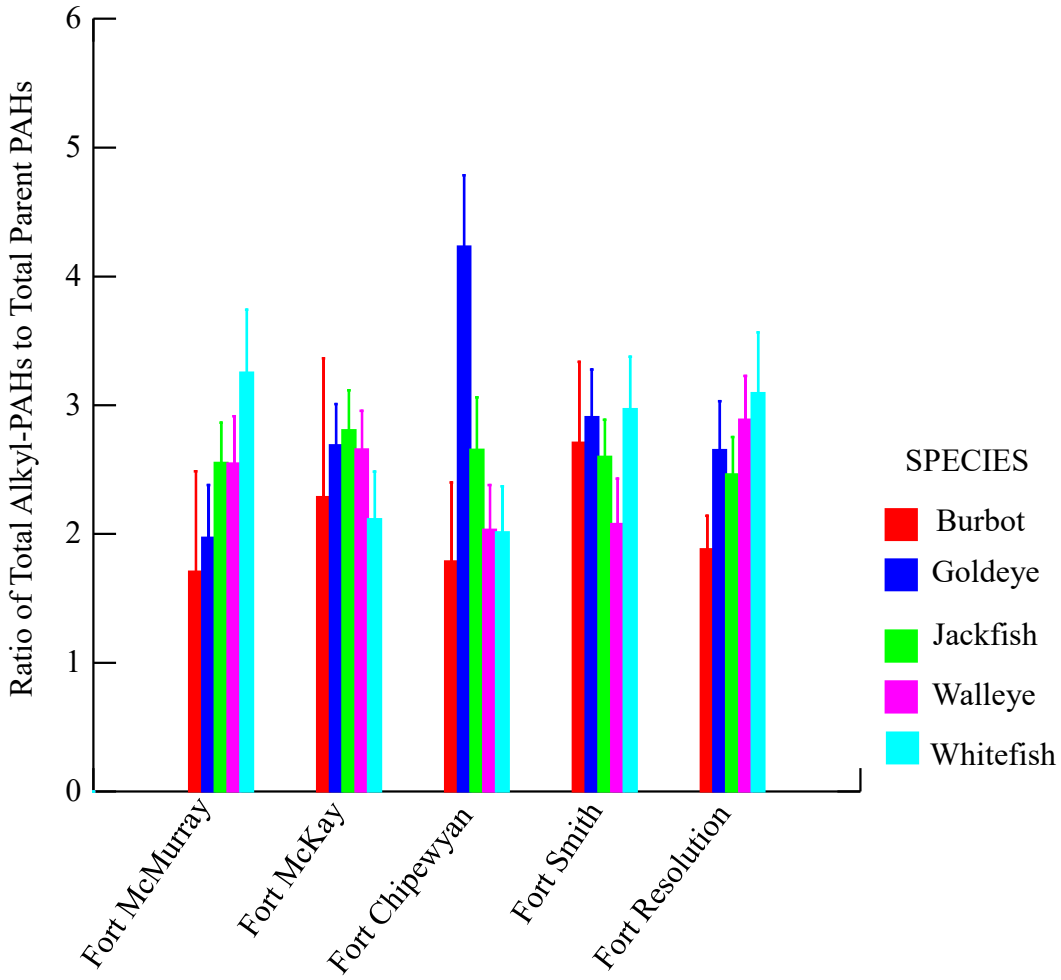


Figure 4.5: Ratio of total alkyl-PAHs to total parent PAHs by species at the locations along the Slave and ARs.

4.3.2 Profile Analysis of LMW and HMW PAHs

Parent PAHs could be classified as low molecular weight PAHs (LMW-PAHs; 2- and 3-ring PAHs) and compared to high molecular weight PAHs (HMW-PAHs; 4-6-ring PAHs) and used as an acceptable method of source identification. The concentration of parent PAHs in fishes from the AR and Slave Lake at different sampling sites and seasons are listed in Table 3.3. Mean concentrations for all species, locations, and seasons of ∑2-ring (Naphthalene), ∑3-ring (Acenaphthylene, Acenaphthene, Fluorene, Phenanthrene, and Anthracene), ∑4-ring (Fluoranthene, Pyrene, Benz(a) anthracene, and Chrysene), ∑5-ring PAHs (Benzo(b) fluoranthene, Benzo(k) fluoranthene, Benzo(a) pyrene, and Dibenz(ah) anthracene), and ∑6-ring (Indeno(1,2,3-cd) pyrene and Benzo(ghi) perylene) PAHs were 5.8, 10.7, 7.2, 4.6 and 1.5 ng/g, wm, respectively. The detected concentration ranged from 1.7 to 81 ng/g, wm for 2-ring PAHs, from detection limit to 43 ng/g for 3-ring PAHs, from detection limit to 73 ng/ml for 4-ring PAHs, from detection limit to 26 ng/g for 5-ring PAHs, and from detection limit to 26 ng/g for 6-ring PAHs. The 16 US EPA priority PAHs were widespread in the sampling location, and predominant in fishes from the AR (Fig. 4.6). The PAH concentration followed the pattern observed for other fishes (Soclo et al., 2000, Nkpaa et al., 2013, Al-Yakoob et al., 1994, DouAbul et al., 1997). 2- and 3-ring PAHs dominated the distribution at all sampling sites, species and seasons, which accounts for 19.4% and 36.2% of ∑PAHs, respectively. Naphthalene was the compounds most accumulated. Phenanthrene is a principal PAH component for petroleum products and a second most prevalent compound in this study (∑178.8 ng/g). Chrysene is normally produced through combustion and was present at a mean concentration of 1.8 ng/g wm.

The ratio of LMW-PAHs to HMW-PAHs observed in the five species, and seasons from the sampling locations were > 1. The mean ratio of LMW-PAHs to HMWPAHs was 2.63, indicating that the total PAHs in fishes from the Athabasca and Slave Rivers originated mainly from petrogenic source (Rocher et al., 2004). The ratio of major combustion-specific PAHs (∑COMB including Fla, Pyr, Baa, Chr, BbF, BkF, BaP, and Inp) to the total concentration of PAHs (∑COMB/∑EPA-PAHs) was 0.411, which eliminates pyrogenic source as the primary source for the measured PAHs and invariably confirms petrogenic source (Stogiannidis and Laane, 2015). There were no significant differences in concentrations of ∑LMW PAHs/HMW PAHs in muscle by species for location and season (Table 4.4). In general, the ratio of LMW-PAHs to HMW-PAHs were greatest in Fort McMurray and Fort Resolution. The relative ratio was greatest in jackfish from Fort Chipewyan (Fig. 4.7). In addition to the diagnostic ratios between PAHs and its alkylated homologues, Principal component analysis (PCA) was performed on the data set shown in Table 4.2 to identify possible statistically independent source tracers. The PCA plots suggest which of the fish have similar patterns of PAH compounds. 425 fish samples were used to perform a Principal Components Analysis (PCA). The first two principal components account for the largest possible variance in the data at 33% and 14% of the total variance, respectively. The third component takes into account only 8% of the total variance and each succeeding component explains the remaining variance as possible. A cross-plot of the scores of the significant components reveal clusters in the data with similar compositions. Figure 4.8 indicates that the variability is largely evenly spread over the entire system.

Table 4.4: Kruskal-Wallis non-parametric one-way analysis of variance test showing differences within species for all sites and seasons using the ratio of LMW-PAHs to HMW-PAHs.

Species	Location		Season	
	Statistic	p-Value	Statistic	p-Value
Whitefish	14.91	0.005	11.92	0.003
Jackfish	6.67	0.154	6.86	0.032
Walleye	6.48	0.166	1.35	0.509
Goldeye	3.14	0.535	2.53	0.28
Burbot	3.02	0.553	7.93	0.019

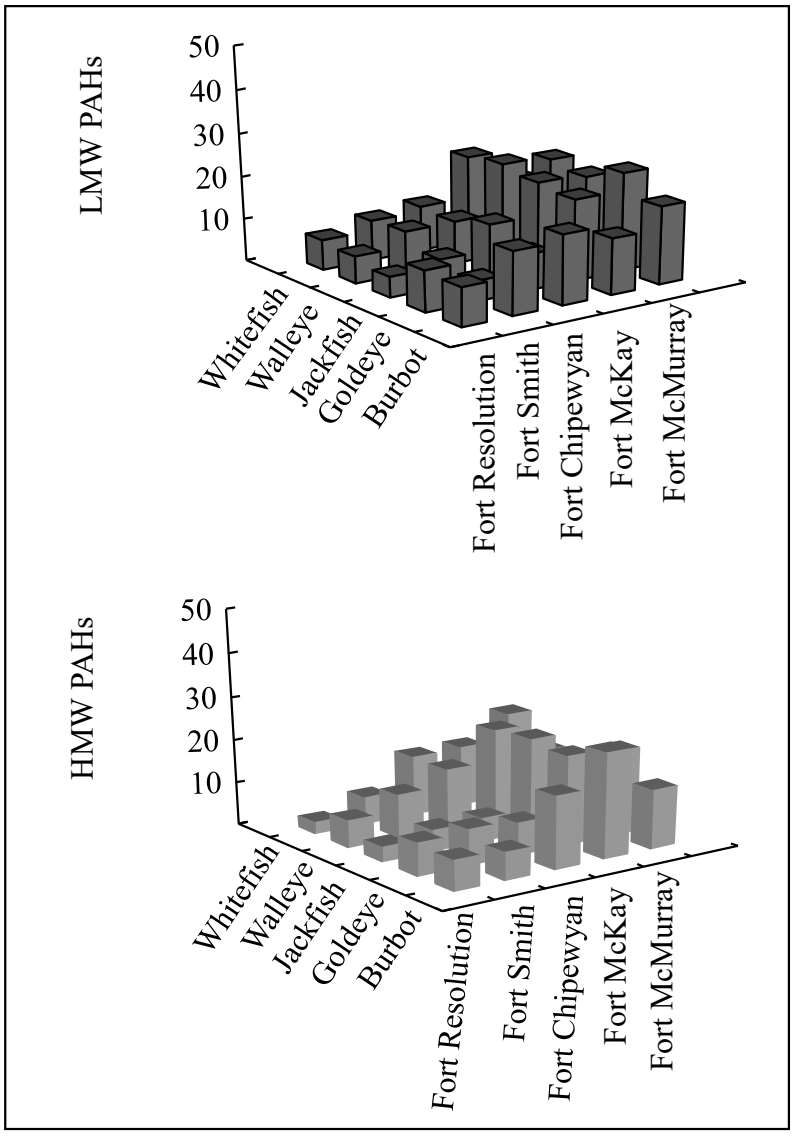


Figure 4.6: Concentrations of low molecular weight (LMW) PAHs and high molecular weight (HMW) PAHs in fish muscle by species from the Slave and ARs.

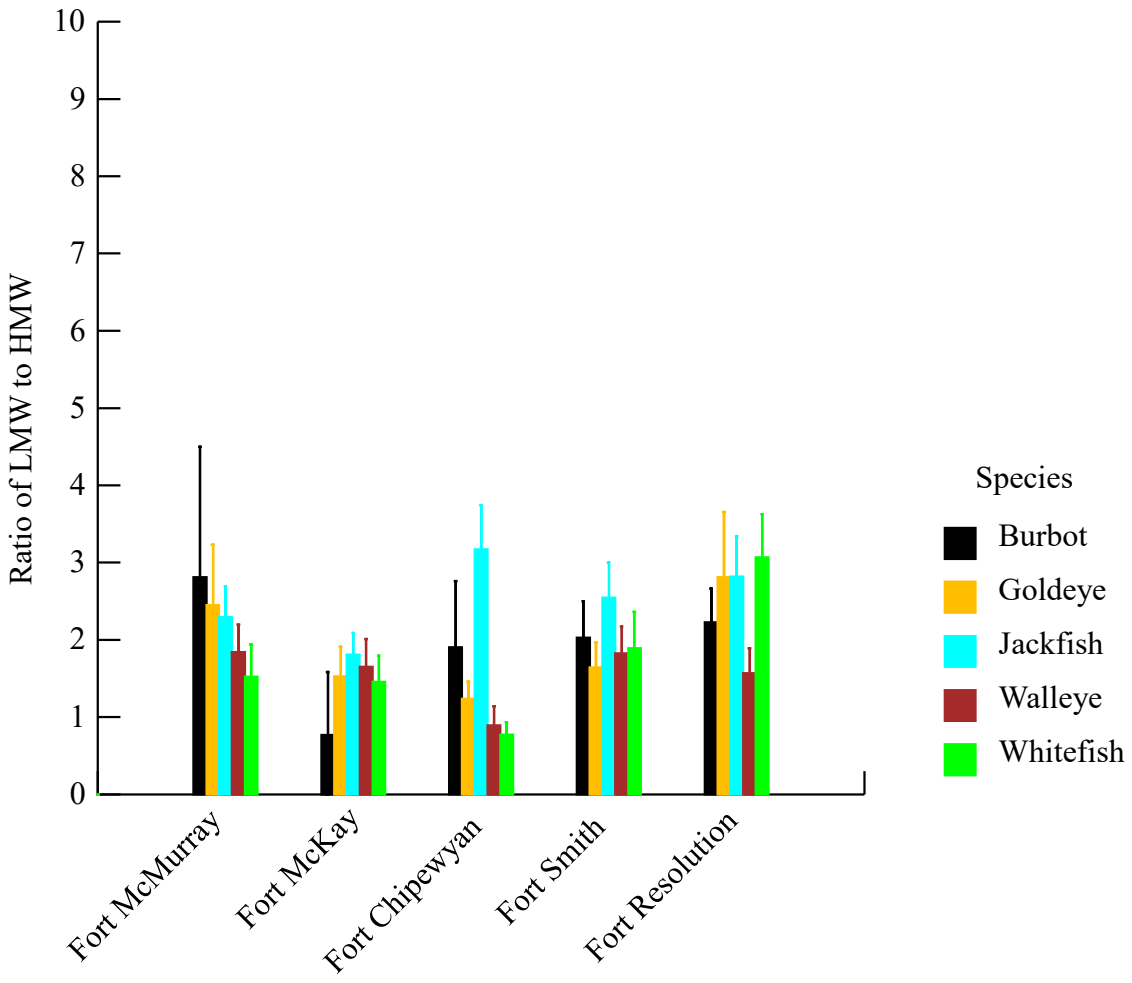


Figure 4.7: Ratio of low molecular weight (LMW) PAHs to high molecular weight (HMW) PAHs by species at locations along the Slave and ARs

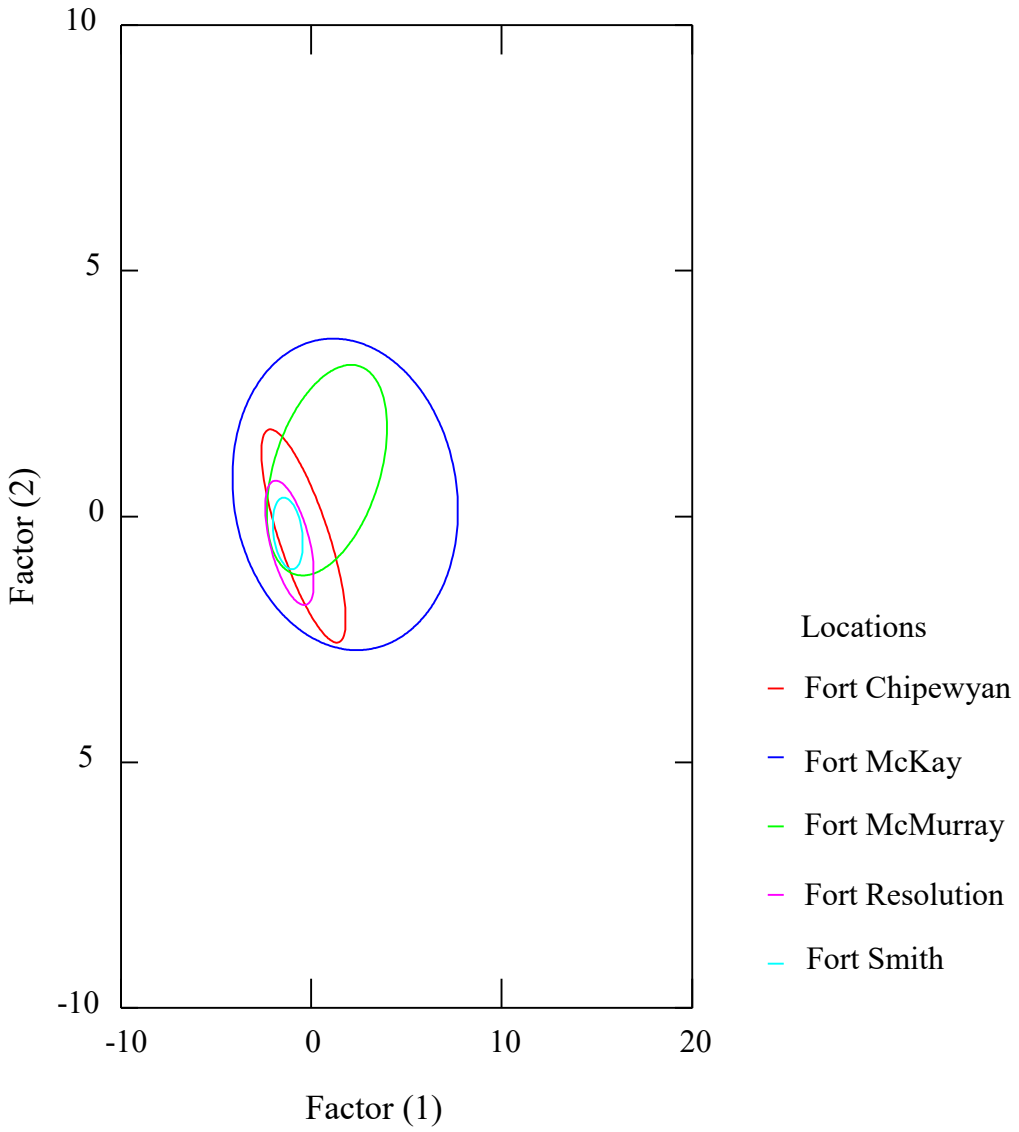


Figure 4.8: Principal components 1 and 2 for fish sample data from the Athabasca and Slave Rivers. The first two principal components account for the largest possible variance in the data at 33% and 14% of the total variance, respectively. The third component takes into account only 8% of the total variance and each succeeding component explains the remaining variance as possible. The data used to run PCA were untransformed alkyl-PAH concentrations.

4.4 Discussion

It is difficult to evaluate alkyl-PAHs in fish samples due to the potential analytical complexity and relatively high rate of PAH depuration in fish. However, chemical fingerprinting, especially of the relative composition of parent PAHs and their alkylated homologue can be used for source identification (Yunker et al., 2002; Pampanin and Sydnes, 2013). PAH compounds from petrogenic exposure often contain petroleum-specific alkylated homologues (C_1-C_4) on the aromatic carbons of naphthalene, phenanthrene, fluorine and chrysene groups (Stogiannidis and Laane, 2015). For example, in an experiment downstream of produced water discharges from an oil platform, caged mussels accumulated greater concentrations of alkyl-naphthalenes and alkyl-phenanthrenes, than their respective parent compounds (Ruus et al., 2006). In contrast, the relative abundance of parent PAHs to their alkylated analogues is indicative of pyrogenic or biogenic sources (Headley et al., 2001).

For centuries, natural oil seeps and forest fires have released PAHs into the AR (Wiklund et al., 2010). The resulting PAH concentration has been reported as a naturally occurring "background concentration" (Akre et al., 2004). PAH background in the Athabasca and Slave Rivers are highly variable and site dependent due to the variable effects of dilution and transport processes within the river system. As such, there is no threshold concentration to quantitatively discriminate natural from anthropogenic PAH input. But qualitative approaches can be utilized. Petroleum PAHs exhibit a bell-shaped pattern within the alkylated groups (Headley et al., 2001; Sauer et al., 1993; Stogiannidis and Laane, 2015). For the fish samples investigated in this study, the distribution of the 2- and 3- ring alkyl-PAHs were generally bell shaped as illustrated in Figure 4.3, characteristic of a petrogenic profile.

The alkylated homologue was greater in concentration for 4-ring alkyl-PAH than their parent compound, albeit slightly for chrysene. In the Phenanthrene/anthracene group, P_3 and P_4 were greater in concentration than the parent compounds. Petrogenic and pyrogenic sources might have contributed to the observed concentrations in the 4-ring PAHs. The delineation however is unclear in biota because alkylated PAHs, regardless of the source, are generally less biodegradable than the parent compounds (Lin et al., 2015). Rapid metabolism could also influence the shape of the alkylation profile and result in a lesser concentration of parent and alkylated PAHs in the muscle samples (Ahokas and Pelkonen, 1984).

Naphthalene was the most prevalent compound, contributing the greatest proportion of the total PAH budget, characteristic of PAH mixtures generated by petrogenic pollution (Hsu et al., 2015; Pampanin and Sydnes, 2013; Stogiannidis and Laane, 2015; Yunker et al., 2002). Headley and Akre, (2001) reported the concentration of PAHs and their alkylated analogues for sediments taken from selected tributaries in the oil sands region of the AR Basin and found the C_0-C_4 series of naphthalenes to be predominant in the total PAH concentration. The greater concentrations of naphthalenes and their alkylated homologue in this study is consistent with studies in other parts of the world from petrogenically impacted environments with similar biological matrices (Al-Yakoob et al., 1994; DouAbul et al., 1997). Many variables including naphthalenes' lower particulate affinity and relatively greater water solubility compared to the higher molecular weight PAHs, might be factors in the observed increase (Cailleaud et al., 2007; Karlsson and Viklander, 2008). The ecology of fish could also be a major factor leading to the elevated presence of naphthalene in this study. Naphthalenes, like other LMW PAHs, tends to be enriched in the fine sand fraction, whereas HMW PAHs are enriched in the fine silt fraction (Karlsson and Viklander, 2008; Mitra et al., 1999). The Athabasca oil sands are dominantly very

fine grain (62.5-250 micrometer) with few coarser sands (Massop, 1980). Considering the ecology of the fish sampled, their exposure to naphthalenes in the fine sand fraction in their habitat is likely.

The presence of LMW PAHs, including naphthalene, in environmental samples is generally associated with un-weathered petroleum (Headley et al., 2002). Weathering, due to a combination of evaporative and biotic losses, causes significant changes to the chemical profile of source oil, resulting in losses of LMW PAHs, leaving only large unresolved complex mixtures with HMW PAHs (Akre et al., 2004; Fedorak and Westlake, 1981 Sauer et al., 1998). The relatively high concentration of naphthalene and its alkylated analogues in this study suggests un-weathered petroleum as a possible source of contamination. It is likely that fish bioconcentrate PAHs from sediments and biota that contain relatively fresh oil (Stout et al., 2001b). This is plausible as parts of AR and its tributaries are incised into the oil sands deposits, causing surface exposure. Besides the bleeding of bitumen from the exposed surface, erosion of the incised portion contributes hydrocarbon related contaminants to the surrounding waters. Oil sands development in the area is also a major pathway of fresh petroleum related contaminants into the AR (Kelly et al., 2009; Zhan et al., 2016)

The distribution of low and high molecular weights PAHs are useful tools for discriminating the petrogenic/pyrolytic origin of PAHs. The higher the LMWPAH/ HMWPAH ratio (greater than 1), the higher the prevalence of petrogenic to pyrogenic source (Pampanin and Sydnes, 2013; Stogiannidis and Laane, 2015). Fig.4.6 shows that LMW PAHs are predominant over HMW PAHs, suggesting a definitive petrogenic origin of PAHs. The average LMWPAH/HMWPAH ratios of fish sampled in Fort McMurray and Fort McKay were 4 and 5, respectively. In contrast, this ratio was 3 in Fort Chipewyan. The ratio in Fort Smith and Fort

Resolution were 5 and 4, respectively. LMWPAH/HMWPAH average ratio of 4 was calculated for the total fish sample. Though the ratio was the same in Fort Smith and Fort Resolution as it was in Fort McKay and Fort McMurray, the absolute concentration was lower in the SR.

The use of multivariate analyses allows us to make reasonable hypotheses about possible similarity of the source of PAHs. In a cross-plot of principal components, samples that have similar compositions are close to each other. When PCA is applied to the data generated from this study, it can be seen that the majority of the samples appear to be grouped together and there are no major sub-groupings, suggesting that the samples are from a similar source (Fig. 4.8). This distribution pattern was observed across the sampling location. Although there are no visible oil sands deposit in Fort Chipewyan, Fort Smith and Fort Resolution, it is possible that river flow downstream is bringing in fresh organic material. Fish migrating from the McMurray formation area could also be a possible source of petrogenic PAHs. Further analysis of the PCA indicates that PAHs in Fort McMurray and Fort McKay have a compositional pattern that is slightly different from samples collected downstream in Fort Smith and Fort Resolution. Minor differences observed may reflect variability due to metabolism in the fishes or variability due to weathering. Thus, some heterogeneity is expected. Hierarchical cluster analysis showed elevated presence of phenanthrene in Fort McKay, strengthening the hypothesis of a definitive petrogenic contribution to PAHs (Gui-Peng, 2000; Magi et al., 2002; Soclo et al., 2000). Petroleum usually exhibits higher phenanthrene concentrations because of its thermodynamic stability (Luca et al., 2004). Similarly, the presence of alkyl-fluoranthene-C3 enriched source in Fort McMurray shows a strong petrogenic character as well (Luo et al., 2008).

Alkyl-PAH concentrations measured in fish muscle from the southern locations are greater than those from the northern locations (Figs. 4.3). Alkyl-PAHs were greatest in Fort

McKay, while the cause of the increase is unclear, it is likely due to many variables including increased soil erosion from the river banks and greater rainfall/runoff events in spring which would have resulted in higher input of oil sands material into the river. Groundwater may also contribute parent and alkylated PAHs from underground oil deposits to the surface water. The greater concentrations could also be indicative of an increase in localized input of fresher oil sands materials or possible emission from development activities in the region (Parajulee and Wania, 2014). The least concentrations observed in Fort Resolution may be as a result of longitudinal dilution along the watershed as well as reduced levels of human activity in these more northern areas. The SR, relative to the AR, is undeveloped, and as such, a gradient is a priori expected to exist in the chemical parameters along the length of the basin. Fallout from forest fires might contribute to the measured concentration of alky-PAHs, especially in samples from Fort Chipewyan, Fort Smith and Fort Resolution. Forest fires are prevalent in the forests around Fort Chipewyan and Fort Smith. However, further analysis would be required to confirm the presence of wood combustion markers such as 1,7-dimethylphenanthrene, perylene and retene (Hsu et al., 2015Stout, 2007; Yunker et al., 2002).

4.5 Conclusion

This study is the first comprehensive assessment of alkyl-PAHs in fish muscle samples from the Athabasca and Slave Rivers. Gas chromatography mass spectrometry analysis of fish muscle confirms that the sampled fish contain measurable levels of alkyl-PAHs. The alkyl-PAH profiles show the substantial predominance of low over high molecular weight (LMW) alkyl-PAHs in samples. I conclude that the underlying reason for the increase of LMW alkyl-PAHs is that the sampled fishes were exposed to un-weathered petroleum dominated source. Fort McKay

had the greatest concentration of parent PAHs and their alkylated homologue. The PAH concentrations rapidly decline downstream to values of typical of remote pristine areas (Headley et al., 2001). This reflects high dilution of water/sediments from upstream. The general presence of naphthalenes and phenanthrenes and the evaluation of molecular ratio (i.e., LMW/HMW alkyl-PAHs) allow us to conclude that in general, the pollution is petrogenic, probably due to increases in oil sand operations around Fort McMurray and Fort McKay. This study helps to fill the knowledge gap regarding the current levels of exposure of alkylated PAHs in fishes from the Athabasca and Slave rivers. Further monitoring of PAH concentrations in the Athabasca and Slave rivers over a longer time period would be needed to establish temporal trends.

Preface to Chapter 5: Health Status of Fishes from the Athabasca and Slave Rivers, Northern Canada

Since the Athabasca and Slave Rivers are recipients of chemical compounds from a variety of sources, fish can provide indications of concentrations of contaminants over time because of their wide distribution, movements in the basins and their key positions in food webs. My fourth objective was to perform fish health investigation by collecting morphometric health measures and perform a systematic assessment of the occurrence of lesions in the fishes. I assessed the health status of economically and culturally significant fishes of nutritional, commercial and recreational significance to residents of the Athabasca and Slave Rivers, Canada.

I recently sent this chapter for publication in Environmental Pollution, under joint authorship with Brett Tendler (University of Saskatchewan), Erin Kelly (Government of the Northwest Territories), John P. Giesy (University of Saskatchewan), and Paul Jones (University of Saskatchewan). I played a leading role in the research design and sample collection and data analysis, Mr Tendler assisted with sample collection. I wrote and revised this manuscript with editorial comments from the other authors. Dr. Kelly was responsible for coordination with communities, the government of NWT and sponsors. Dr. Giesy provided part of the research funding and editorial feedback. Dr. Jones provided a substantial part of my student and research funding. Furthermore, he coordinated the field work, assisted with experimental design, data interpretation and provided editorial feedback.

CHAPTER 5

Health Status of Fishes from the Athabasca and Slave Rivers, Northern Canada

Abstract

This work evaluated the health status of economically and culturally significant fish in the Athabasca and Slave Rivers, Canada and the potential impact of industrial development on them. This study concentrated on species of nutritional, commercial and recreational significance to residents of the Athabasca and Slave Rivers. Sampling targeted thirty (30) individuals each of whitefish (*Coregonus clupeaformis*), jackfish/northern pike (*Esox luscius*), walleye (*Sander vitreus*), goldeye (*Hiodon alosoides*) and burbot (*Lota lota*) in seven locations including Fort McMurray, Fort McKay, Fort Chipewyan, Fort Smith and Fort Resolution during spring, summer, fall, and winter over five years, between 2011 and 2015. A total of 1901 individual fish were collected including 428 goldeye, 409 whitefish, 378 jackfish, 514 walleye and 120 burbot. Lesions and other abnormalities were encountered in the sampled fish but their frequency was relatively low and uniform across locations. A resurgence in condition factor of all species after a low in 2011 was observed. Annual variation was also observed in condition factor and the incidence of anomalies. Morphometric data demonstrated relatively consistent fish health in both the Athabasca and Slave rivers. Analysis of condition factor and somatic indices did not demonstrate consistent impacts along the river system. Overall, the health of fish does not appear to be adversely affected by the current level of development in the Alberta oil sands region.

5.1 Introduction

The AR basin supports pulp and paper industries, agriculture, forestry, and the Athabasca Oil Sands Region (AOSR), which is located just downstream of Fort McMurray (MRBB, 2003c). In recent years, oil sands deposits have been exploited as a source of energy (Government of Alberta, 2013), which has resulted in accumulation of an estimated trillion liters of oil sands affected process water (OSPW) in tailings ponds. The concentration of industrial infrastructure, ore-mining, bitumen extraction, and bitumen upgrading and liquid waste discharges (tailings ponds) adjacent to the AR (AR) have raised concerns about the aquatic eco-toxicological risk of oil sand related activities (Dowdeswell et al., 2010; Kelly et al., 2010). Potential effects of these activities could potentially be observed down the length of the AR (AR) and eventually the Slave River (SR) into which it discharges (Schwalb et al., 2015). The AR originates in the eastern Rocky Mountains of Alberta, Canada near Jasper National Park and flows in a northeast direction to Lake Athabasca. The AOSR contains several towns, including Fort McMurray, Fort McKay and Fort Chipewyan. The SR originates where the Athabasca and Peace River sub-basins converge to form the Peace-Athabasca Delta, the SR then flows north into the Northwest Territories. The waters then flow northwards to the Mackenzie River via the Great Slave Lake, eventually emptying into the Arctic Ocean (RAMP, 2012). The SR contains a few small settlements (including Fort Smith and Fort Resolution). As a northern river basin, this ecosystem is under increasing pressure due to development and changes in climatic conditions with potential influences on water quality and ecological integrity (Dube and Wilson, 2013). Since the Athabasca and Slave Rivers are recipients of chemical compounds from a variety of sources, valued ecosystem components including fish can provide indications of concentrations of contaminants over time because of their wide distribution, movements in the basins and their key positions in food webs (Mill et al., 1997).

The Athabasca and Slave Rivers provide habitat for fish with cultural, subsistence, commercial and recreational values (Nelson and Paetz, 1992). The basin supports a rich assemblage of forty species of fishes, thirty-five of which utilize the AR and twenty-five the SR for spawning and feeding (Mill et al., 1997). Fishes that live in the Athabasca and Slave Rivers are adapted to habitat fluctuations with life history strategies that permit them to survive variability through the annual cycle of seasonal change in temperature, hydrological regime, turbidity, contaminants and availability of food (Schwalb et al., 2015).

There are a number of potential stressors present in the Athabasca and Slave Rivers watershed, including forestry, urban development, municipal sewage discharge, recreational activities and oil sands development that could potentially impact life history traits, including size, health and reproduction of populations of fish (Brown et al., 1996; Schwalb et al., 2015). These traits vary in relation to environmental conditions, a change in one trait might cause changes in other traits. Fish health may continue to degrade due to changes in habitat unless preventative and restorative strategies to achieve health and integrity are in place. Some stressors in the AR, including natural deposits of bitumen are a source of polycyclic aromatic hydrocarbons (PAHs), and trace metals and metalloids that have chronic, and sometimes acute, effects on fish health (Lin et al., 2015). Oil sands operations produce large volumes of process-affected water (OSPW) containing solids, inorganic ions, naphthenic acids (NAs), alkylated polycyclic aromatic hydrocarbons (PAHs) and heavy metals (Colavecchia et al., 2004; Van den Heuvel et al., 1999). Human activities can result in changes in dissolved concentrations of oxygen and changes in water levels and flows. Reduction of oxygen has been known to cause reduced development time in fish eggs, physiological stress in adult fish, and fish mortality

(Squires et al., 2010). It is necessary to evaluate the status of fish in the rivers and to assess the potential impacts of industrial development on them.

Analyses of physiological responses of Athabasca and Slave Rivers fish to stresses is reported by Brown et al., (1993 &1996) and by Gibbons et al., (1995). The main objective of the current study was to collect detailed information about the health of selected fishes in the Athabasca and Slave Rivers. This was the first time that inter-seasonal comparison of condition factor of fishes has been systematically examined in several locations on the Athabasca and Slave Rivers. The study was designed to include specific measures of gross external and internal pathology, including external abnormalities like tumors, lesions, scars or injuries, skin discoloration, deformities and parasites. Nearly 2000 fish were examined for morphometric and abnormalities. Lake whitefish, northern pike, burbot, walleye and goldeye were the species examined. These species where chosen mostly due to their prevalence in the sampling locations (i.e., patterns of migration and habitat of spawning of different fishes), their cultural and nutritional significance to communities in the area, and their dietary strategy such as benthic, supra-benthic, or pelagic and, trophic status. In order to increase our understanding of the effects of exposure of contaminants to the sampled species we used condition factor (CF). Fish condition is a well-known indicator of health in individual fish or populations, giving an indication of feeding activity and accumulated energy reserves during the previous few weeks (Gauthier et al., 2009; Schwalb et al., 2015). Gonadosomatic index (GSI) and hepatosomatic index (HIS) were measured as estimators of accumulated energy reserves for reproduction, maintenance or expenditures that do not contribute to somatic growth during the previous few weeks. Locations, ranging from those upstream of or remote from sources of contamination to

possibly more contaminated (Kelly et al., 2009; Ohiozebau et al., 2015), were selected along the Athabasca and Slave Rivers.

5.2 Materials and Methods

5.2.1 Sampling

A detailed description of methods of collection of fishes and sampling methodologies is provided in section 1.7 (Sample collection). Briefly, fish were collected in cooperation with First Nations fishers, and regional and federal agencies. Efforts were made to collect thirty (30) individuals each of whitefish (*Coregonus clupeaformis*), jackfish/northern pike (*Esox luscius*), walleye (*Sander vitreus*), goldeye (*Hiodon alosoides*) and burbot (*Lota lota*) in locations near Fort McMurray (FMU, 56^{o}N, 111^{o}W), Fort McKay (FM, 57^{o}N, 112^{o}W), Fort Chipewyan (FC, 58^{o}N, 111^{o}W), Fort Smith (FS, 60^{0}N, 111^{0}W) and Fort Resolution (FR, 61^{o}N, 113^{o}W) in June/July (summer) 2011, October (fall) 2011 and May (spring) 2012. Fish were sampled in both rivers in 2011 and 2012 however funding was not available for continued sampling in the AR after 2012. Although the five stations continued to be sites in the AR and SR from which fishes were collected (Fig 1.3), the need was recognized for an up stream, reference location on the AR that was isolated from oil sand deposits and operations, and two locations on the SR near Fort Fitzgerald (FF, 59^{0}N, 11^{0}W) and the Peace river at Peace Point (PP, 60^{o}N, 112^{o}W). Consequently, whitefish, jackfish, walleye, goldeye and burbot were sampled up-Stream (US) of Fort McMurray (FMU), and at Fort Fitzgerald (FF) and Peace Point (PP) during the May (spring) 2012 collection. Fish collected in June/July (summer) between 2013 and 2015 in locations near FS and FR included walleye, jackfish and whitefish. Burbot were collected in Dec of 2011 (winter) in FR and FS where weather and logistics permitted.

Lesions and other abnormalities including lymphocystis and general 'unhealthy' features in fish are classified in this study as 'anomalies'. Identifying anomalies was based on best professional judgement. Minor external asymmetries, healed wounds and haemorrhages of fins and eyes were not considered anomalies but were noted. In general, any unusual external feature not immediately explainable was classified as an anomaly. While this approach might ultimately overestimate physical defects attributable to chemical contamination or other environmental stressors, our main goal in this study was to evaluate relative incidences of anomalies among locations. From this perspective we are confident that the same criteria were applied to all fish examined at all locations, thus ensuring the comparability of incidences between locations.

5.2.2 Fish Condition

Condition factor (CF or 'condition') was calculated as $CF = (M_f/L^3)100$ (where "M_f" is fish mass, "L" is the fish length). Gonadosomatic index (GSI) was calculated as $GSI = (G_f/M_f)100$ (where "G_f" is gonad mass of fish, "M_f" is the fish mass). And Hepatosomatic index (HIS) was calculated as HSI = (Lf/Mf)100 (where "L_f" is liver mass of fish, "M_f" is the fish mass).

5.2.3 Statistics

The Spearman Rank Correlation Coefficient was used to investigate associations between variables. Significance levels were set according to the following probability ranges: $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$. All data were tested for normality by use of the Shapiro-Wilk test. Conditions for ANOVA were not fulfilled i.e., the assumption of normality of the distributions of the residuals were violated. Therefore, nonparametric Kruskal-Wallis test was used to test for differences

between the fish species, locations, and seasons. All statistical analyses were conducted using SYSTAT version 13.1 (SYSTAT, 2009).

5.3 Results

Biometrics of fishes sampled for this study are reported in Table 5.1. A total of 1901 individual fish were collected and included 428 goldeye, 409 whitefish, 378 jackfish, 514 walleye, and 120 burbot. The mass and length of burbot ranged from 154 g (FC, summer, 2011) to 3890 g (FS, winter, 2015) and 28.6 cm (FC, summer, 2011) to 73.7 cm (FS, winter, 2015), respectively. Female burbot were significantly greater in length and mass in the SR than in the AR ($P< 0.001$). The physical characteristics of male burbot did not differ significantly ($p> 0.05$). Goldeye ranged from 43 g (FF, spring, 2012) to 1281 g (FF, spring, 2012) and 6.35 cm (FS, summer, 2011) to 45.7 cm (FS, spring, 2012) for mass and length, respectively. Female goldeye exhibited significant difference in length and mass across location ($p < 0.001$). Physical characteristics of male goldeye did not differ significantly ($p > 0.001$). Mass and length of jackfish ranged from 121 g (FMU, summer, 2011) to 9346 g (FF, spring, 2012) and 26.0 cm (FMU, summer, 2012) to 104.1 (FF, spring, 2012), respectively. Physical characteristics of female and male northern pike did not differ significantly across locations ($P > 0.001$). Mass and length of walleye ranged from 170 g (FR, spring, 2012) to 5100 g (FF spring, 2012) and 2.4 cm (FM, summer, 2011) to 123.2 cm (FF, spring, 2012), respectively.

Male walleye exhibited significant differences in length among locations ($p < 0.001$). Female walleye from the SR were significantly greater in length and mass relative to those from the AR ($P< 0.001$). Masses of whitefish ranged from 536 g (FC, summer, 2011) to 3111 g (FF,

spring, 2012) and length ranging from 31.8 cm (FS, summer, 2012) to 56.5 cm (FF, spring, 2012). Female whitefish exhibited significant difference in mass among locations ($p < 0.001$). Physical characteristics of male whitefish did not differ significantly across locations ($P > 0.001$).

Table 5.1: Biological parameters, condition factors and somatic indexes of fishes collected from various locations. Number of individual fish collected indicated in brackets (n). US= Up-Stream, FMU= Fort McMurray, FM= Fort McKay, FC= Fort Chipewyan, FF= Fort Fitzgerald, PP= Peace Point, FS= Fort Smith, FR= Fort Resolution.

Species	Location	Length (cm)	Mass (g)	HSI (%)	GSI (%)	CF (%)
Walleye	FMU (58)	45.0 ± 8.0	1,363 ± 665	1.19 ± 0.43	0.71 ± 1.04	1.19 ± 0.27
	FM (68)	45.2 ± 6.3	1109 ± 370	1.17 ± 1.65	1.07 ± 1.65	1.17 ± 0.16
	FC (68)	50.4 ± 5.2	1365 ± 363	1.31 ± 0.41	0.93 ± 1.04	1.05 ± 0.14
	FS	45.7 ± 6.8	1208 ± 508	1.36 ± 0.48	2.99 ± 4.77	1.19 ± 0.20
	FR	45.3 ± 5.8	1280 ± 425	1.28 ± 0.65	1.31 ± 1.16	1.32 ± 0.19
	FF (30)	58.4 ± 13.6	1998 ± 808	1.78 ± 0.48	1.18 ± 2.80	1.06 ± 0.22
	PP (31)	50.6 ± 7.4	1494 ± 798	1.25 ± 032	0.57 ± 0.23	1.07 ± 0.08
Burbot	FMU (34)	38.5 ± 2.6	420 ± 87	5.16 ± 1.86	0.51 ± 0.22	0.72 ± 0.02
	FM (2)	55.2 ± 0.90	1075 ± 7.07	2.05 ± 0.09	7.71 ± 0.43	0.63 ± 0.02
	FC (5)	51.6 ± 13.0	1109 ± 540	3.86 ± 1.28	4.74 ± 3.21	0.71 ± 0.05
	FS (8)	53.0 ± 10.0	1282 ± 827	3.03 ± 1.30	8.56 ± 6.20	0.75 ± 0.14
	FR (68)	62.3 ± 3.9	1870 ± 453	5.71 ± 2.12	5.10 ± 4.06	0.77 ± 0.15
Jackfish	FMU (40)	65.4 ± 17	2522 ± 1493	1.57 ± 0.55	1.84 ± 2.68	0.80 ± 0.19
	FM (29)	67.3 ± 12.9	2474 ± 1440	1.65 ± 0.48	1.34 ± 1.42	0.75 ± 0.11
	FC (58)	67.9 ± 10.1	2495 ± 1508	1.29 ± 0.47	1.20 ± 1.55	0.72 ± 0.16

	FS	65.4 ± 10.2	2377 ± 1159	1.35 ± 0.51	3.26 ± 4.42	0.79 ± 0.13
	FR	67.3 ± 9.18	2610 ± 1305	1.41 ± 0.52	2.44 ± 3.80	0.78 ± 0.14
	FF (30)	77.6 ± 13.0	3853 ± 2111	1.63 ± 0.61	1.16 ± 1.80	0.77 ± 0.10
	PP (15)	65.4 ± 12.5	2165 ± 1414	1.12 ± 0.46	3.88 ± 5.01	0.70 ± 0.12
Goldeye	FMU (62)	34.6 ± 3.7	532 ± 137	1.16 ± 0.22	1.70 ± 1.94	1.27 ± 0.17
	FM (89)	34.5 ± 5.6	532 ± 198	1.32 ± 0.22	3.27 ± 3.97	1.24 ± 0.13
	FC (75)	36.0 ± 3.7	546 ± 131	1.35 ± 0.42	2.74 ± 3.39	1.13 ± 0.12
	FS (87)	33.4 ± 4.9	447 ± 168	1.29 ± 1.08	4.70 ± 5.13	1.70 ± 5.42
	FR (48)	36.2 ± 3.3	576 ± 155	1.51 ± 1.73	6.02 ± 4.71	1.18 ± 0.13
	FF (21)	25.2 ± 6.6	205 ± 290	1.37 ± 0.41	0.86 ± 1.77	0.93 ± 0.14
	PP (30)	37.8 ± 4.0	627 ± 165	1.09 ± 0.21	10.7 ± 4.75	1.12 ± 0.08
	US (13)	35.8 ± 2.6	630 ± 129	1.00 ± 0.29	4.39 ± 6.24	1.37 ± 0.26
Whitefish	FMU (34)	41.4 ± 2.4	1056 ± 235	0.89 ± 0.20	2.66 ± 3.77	1.47 ± 0.22
	FM (63)	41.6 ± 3.6	1191 ± 319	0.90 ± 0.22	4.51 ± 4.90	1.63 ± 0.18
	FC (82)	40.8 ± 4.2	1152 ± 376	1.31 ± 0.44	1.78 ± 3.29	1.65 ± 0.21
	FS (97)	39.2 ± 2.7	932 ± 188	0.85 ± 0.31	4.31 ± 4.62	1.54 ± 0.21
	FR (120)	40.1 ± 3.6	1011 ± 314	1.07 ± 1.20	2.11 ± 2.70	1.53 ± 0.26
	FF (10)	45.4 ± 6.6	1590 ± 829	1.18 ± 0.60	0.62 ± 0.54	1.60 ± 0.22

A test to compare among seasons, within species, showed significant differences ($p < 0.001$) in length and mass of jackfish and walleye. There were no significant differences in length ($p = 0.462$) or mass ($p = 0.003$) for burbot and length ($p = 0.015$) and mass ($p = 0.005$) for goldeye between seasons. Whitefish exhibited significant differences in length ($p < 0.001$) but no significant differences in mass ($p = 0.015$) among seasons.

Condition factors of jackfish and burbot were relatively consistent among sampling locations (Fig 5.1). Female burbot exhibited significantly greater HSI and GSI values in the SR relative to the AR ($P< 0.001$). Male northern pike showed a significantly greater GSI in the SR compared to the AR. The condition of goldeye showed a trend of decreased condition downstream, among locations, but there were certain exceptions to this trend. The condition of goldeye from FC and PP was not different from that of goldeye from FS and FR. Female goldeye exhibited significant difference in HIS, GSI and CF across locations ($p < 0.001$). For whitefish, the condition factor increased between FMU, FM and FC, then remained uniform across FS and FR. Female whitefish exhibited significant differences in HSI and GSI across locations ($p < 0.001$). Male whitefish exhibited significant difference in GSI and CF across locations ($p < 0.001$). Walleye condition decreased from FM to FF, then increased from FS to FR. Male walleye exhibited significant difference in GSI and CF across locations ($p < 0.001$). Female walleye was significantly greater in HIS, GSI and CF in the SR relative to the AR ($P< 0.001$).

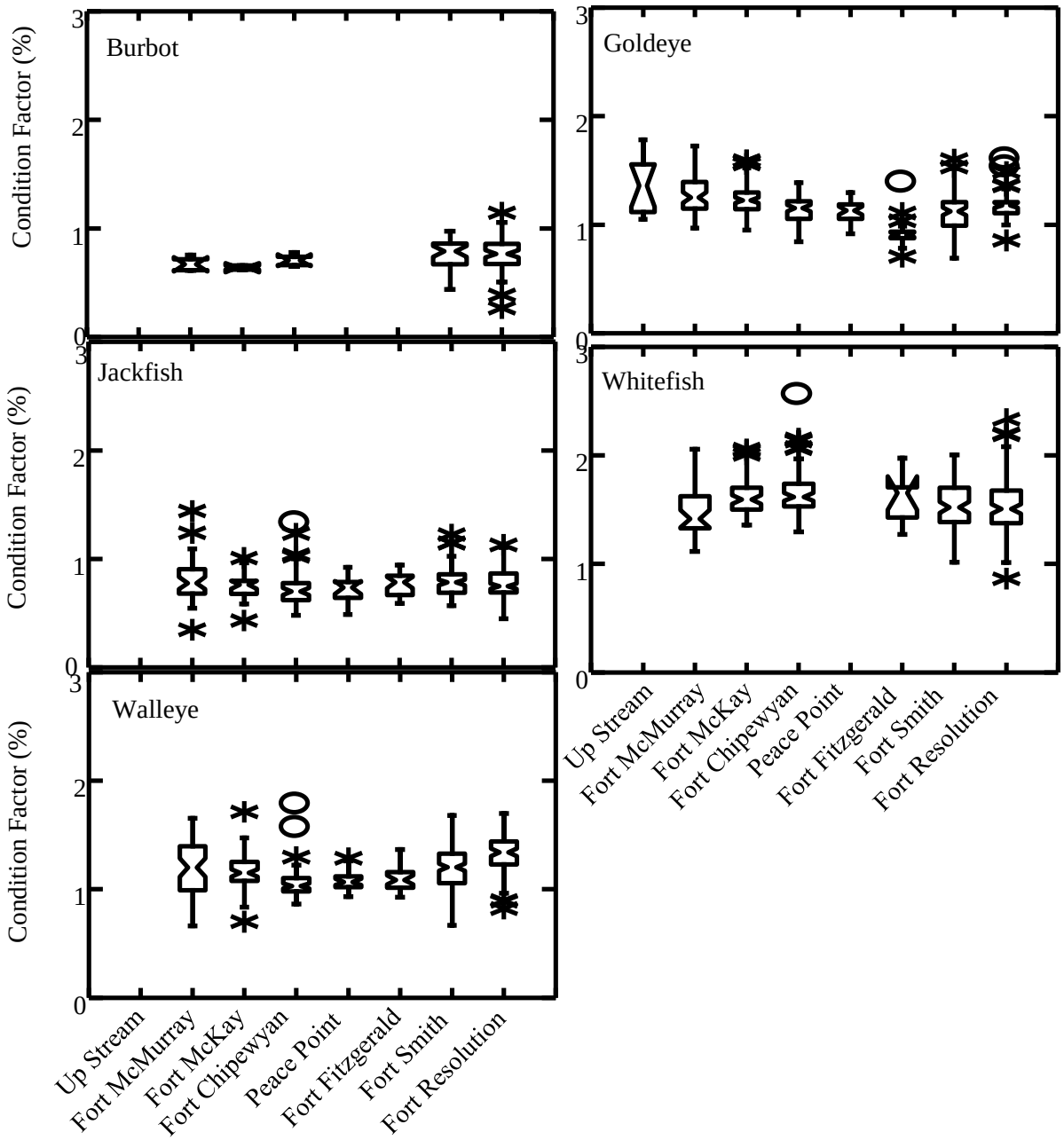


Figure 5.1: Box plots showing the spread of condition factors of fishes sampled from eight locations, during the sampling seasons. Confidence interval is 95%. The width of the box shows the interquartile range. The top 25% concentrations are shown by the top whisker. The circles above the stars indicate outliers more than 3/2 times of upper quartile. These are the combined data for all years.

Inter-annual differences in condition were observed in fishes from locations sampled from 2011 to 2015. In all cases conditions of fishes in 2014 and 2015 were significantly greater than in 2011 ($p < 0.001$) (Fig 5.2 a,b,c). For example, condition of walleye at FS and FR were 42% and 45% greater in 2015 than in 2011, respectively. Similarly, mean condition of burbot at FR was 48% greater in 2015 than in 2011. There was a resurgence of condition of jackfish from 2011 to 2015 at all locations, especially in FS and FR by 44 %, respectively. Condition of whitefish was greater in 2014 than in 2011 by 45% and 40% in FS and FR, respectively. But there were certain exceptions to this trend. Jackfish and whitefish from FC in 2012 spring and fall displayed lower condition than fish from summer 2011.

Relationships between condition of the sampled fishes and other variables including Gonadosomatic index (GSI) and Hepatosomatic index (HSI) were examined (Table 5.2). In walleye and whitefish, during fall sampling, positive relationships were found between GSI and HSI. A similar result was found for burbot caught during the winter. The condition of burbot was correlated with mass of fish caught in fall, spring and winter. During fall sampling, a positive relationship was observed between condition and body mass for jackfish. A similar relationship was found for whitefish collected during summer, fall and spring. Relationships between condition factor and concentrations of PAHs in bile and muscle were also studied, but there were no significant relationships observed at any location for any years ($P > 0.001$).

The incidence of fish with anomalies was <5% of total fish examined (93 of 1901). The greatest incidences of anomalies in fish collected during 2011 were observed at FMU and FC with 9% and 7 %, respectively (Table 5.3 a&b). The greatest incidences of anomalies in 2012 were also observed in FMU and FM with 12% and 8% of fish collected, respectively.

Table 5.2: Relationships between condition factor and other fish biometrics. R values are reported. NS= not significant

Species	Season	Length	Mass	HSI	GSI	Spleen
Walleye	Summer	NS	0.12	0.32	0.19	0.17
	Fall	NS	0.28	0.43	0.43	0.04
	Spring	NS	0.04	NS	0.04	NS
Burbot	Summer	NS	0.27	0.60	0.13	0.35
	Fall	NS	0.54	NS	NS	NS
	Spring	NS	0.42	NS	NS	NS
	Winter	0.23	0.47	0.34	0.42	0.19
Jackfish	Summer	NS	0.23	0.20	0.10	0.10
	Fall	0.21	0.54	0.14	0.12	0.34
	Spring	NS	0.16	0.36	0.11	NS
Goldeye	Summer	NS	NS	NS	NS	NS
	Fall	NS	0.23	0.16	0.11	0.08
	Spring	NS	0.19	0.09	NS	0.07
Whitefish	Summer	NS	0.35	0.22	0.31	0.15
	Fall	NS	0.38	0.34	0.39	NS
	Spring	0.08	0.45	0.17	0.07	0.11

Table 5.3a: Total number of fish collected among locations and seasons.

Year/Location	US	FMU	FM	FC	FF	PP	FS	FR
2011	n/a	115	180	170	n/a	n/a	209	200
2012	13	88	71	122	91	76	86	99
2013	n/a	n/a	n/a	n/a	n/a	n/a	56	53
2014	n/a	n/a	n/a	n/a	n/a	n/a	76	52
2015	n/a	n/a	n/a	n/a	n/a	n/a	87	57

Table 5.3b: Occurrence of anomalies both as absolute numbers (AN) and as percentage incidence rates (%) relative to the total number of fish collected.

Year	US		FMU		FM		FC		FF		PP		FS		FR	
	an	%	an	%	an	%	an	%	an	%	an	%	an	%	an	%
2011	n/a	n/a	7	6	9	5	7	4	n/a	n/a	n/a	n/a	7	3	11	5
2012	1	8	11	12	7	9	9	8	7	8	6	7	4	5	5	5
2013	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	2	3
2014	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	1	1	0	0
2015	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	1	1

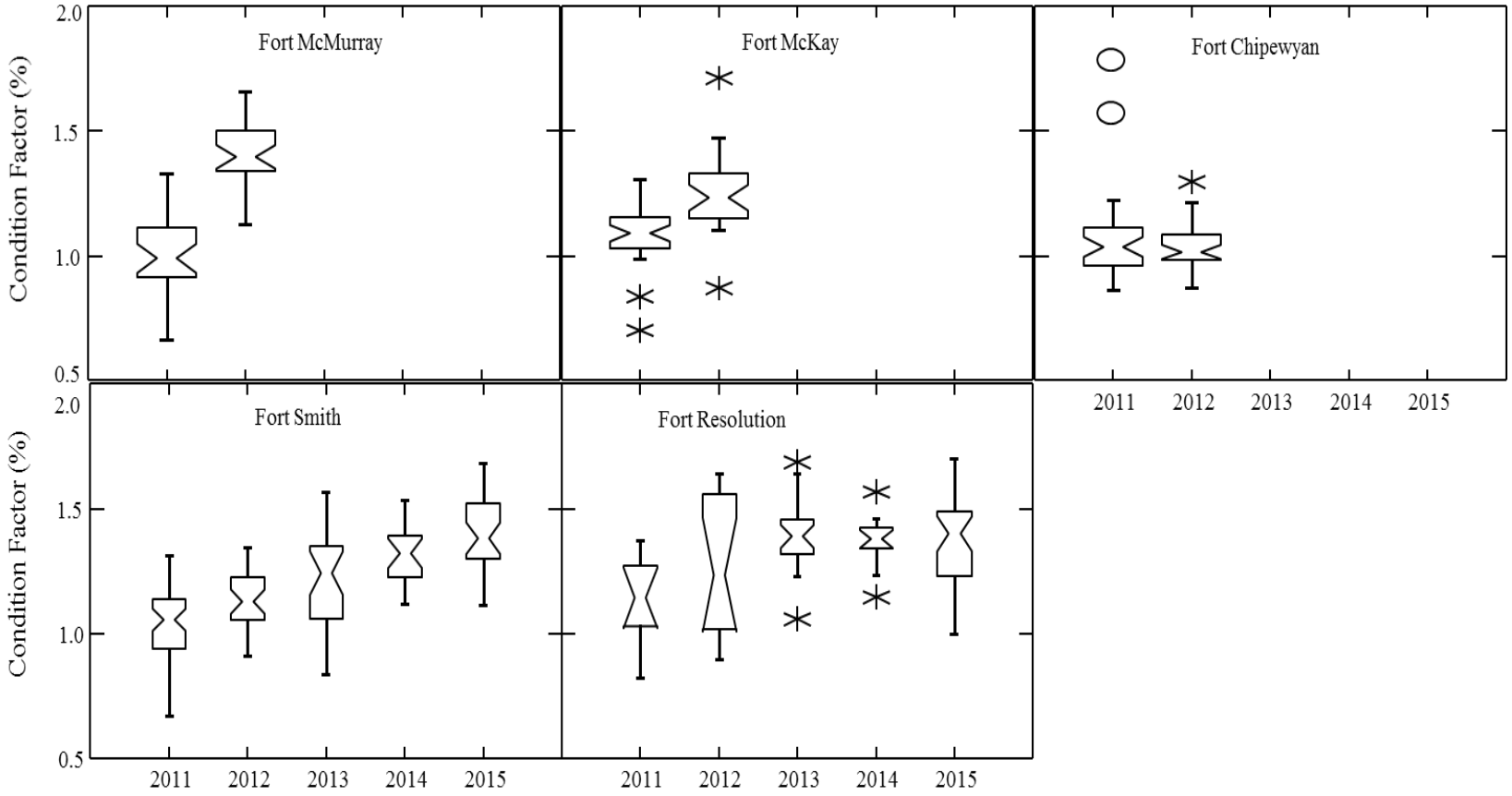


Figure 5.2a: Box plots showing seasonal comparison of condition factor of walleye. Confidence interval is 95%. The width of the box shows the interquartile range. The top 25% concentrations are shown by the top whisker. The circles above the stars indicate outliers more than 3/2 times of upper quartile.

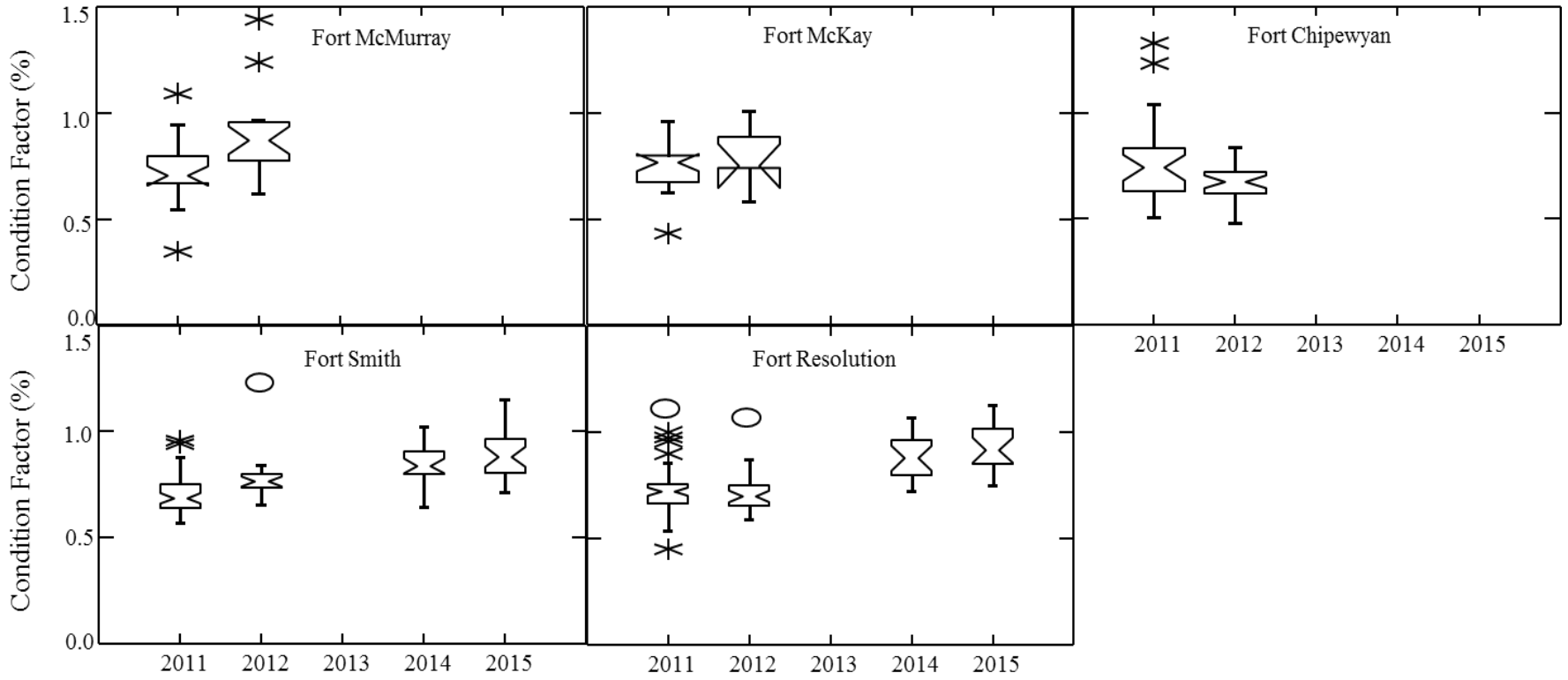


Figure 5.2b: Box plot showing seasonal comparison of condition factor of northern pike. Confidence interval is 95%. The width of the box shows the interquartile range. The top 25% concentrations are shown by the top whisker. The circles above the stars indicate outliers more than 3/2 times of upper quartile.

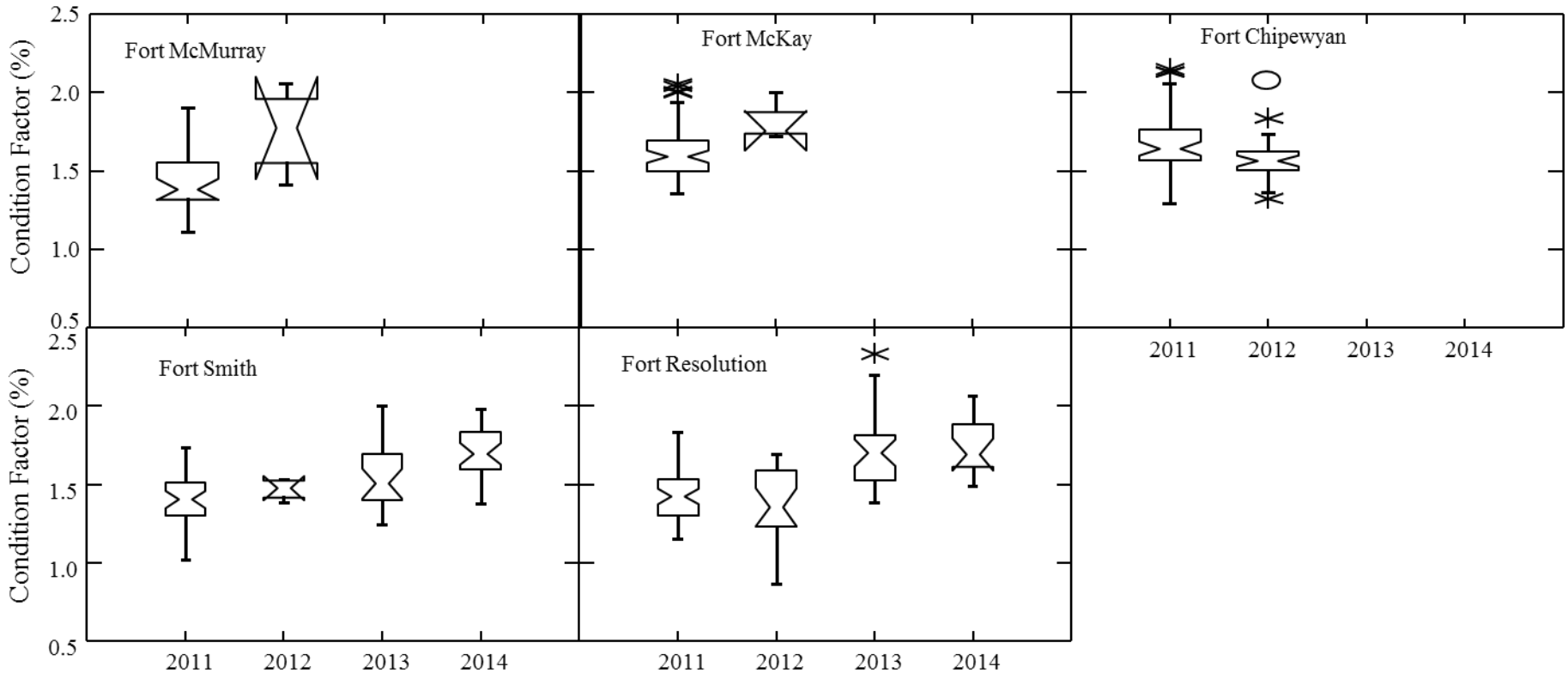


Figure 5.2c: Box plot showing seasonal comparison of condition factor of walleye. Confidence interval is 95%. The width of the box shows the interquartile range. The top 25% concentrations are shown by the top whisker. The circles above the stars indicate outliers more than 3/2 times of upper quartile.

5.4 Discussion

The primary purpose of this study was to determine the health condition of fishes in the Athabasca/Slave River watershed. This study placed special emphasis on individual fish in its effort to evaluate effects of development on the Athabasca and Slave Rivers. We did not conduct studies to determine the population dynamics or population level health of the fish species. Instead, we investigated the physical and biological components of fish in the rivers, and used the condition factor and other morphometric indices, to evaluate overall, relative well-being of fish (Bagenal and Tesch, 1978; Bolger and Connolly, 1989). This emphasis arose in part to address the concerns of local communities relative to the anecdotally increased number of 'deformed' or 'unhealthy' fish caught in their subsistence fisheries.

Several studies have investigated effects of pollutants on condition factor of fishes (Clements and Rees, 1997; Dela Torre et al., 2000; Dethloff et al., 2001). The relationship between body condition and chemical contaminants can be complex (Gauthier et al., 2009 & 2014). For example, contaminants can reduce the abundance of benthic invertebrates, which in turn can affect ability of benthivorous fish to feed (Gauthier et al., 2009; Kovecses et al., 2005; Munkittrick et al., 1991). Conversely, indirect effects of contaminants might improve conditions of fishes by reducing the presence of predators or competitors (Bertolo and Magnan, 2005; Gauthier et al., 2009). Greater concentrations of contaminants, especially metals, in tissues of fishes have been linked to lesser condition (Bervoets and Blust, 2003; Suns and Hitchin, 1990). Living in a contaminated environment can result in increases of expenditures or energy and lead to eventual reduction in condition of fish (Bennet and Janz, 2007; Pyle et al., 2008). In a related study, fishes collected from the AR contained greater concentrations of PAHs in bile and muscle, compared with fishes sampled in the SR (Ohiozebau et al., 2015). It was therefore expected that fishes from

Fort McMurray and Fort McKay would exhibit lesser condition factors relative to fishes from the SR. For example, fishes from Fort McMurray and Fort McKay had greater concentrations of biliary metabolites of PAHs as well as greater concentrations of parent and alkylated PAHs (Figure 5.3). However, no significant relationship was found between concentrations of PAH in bile and CF when fishes were stratified by location (Table 5.4). Plots of condition factor against concentrations of PAHs in bile or muscle did not reveal obvious relationship (Figure 5.4). This result is consistent with those of other studies that investigated the relationship between concentrations of metal or PAH and CF (Bervoets and Blust, 2003; Schwalb et al., 2015). The lack of associations between concentrations of PAHs and metrics of health in fishes is most likely due to the relatively small concentrations of contaminants. The condition factor remained relatively uniform in fish from Fort McMurray and Fort McKay, increased slightly at Fort Chipewyan, moderated at Fort Smith to values observed upstream before an increase at Fort Resolution. The increase in condition factor downstream might be due to build-up of energy in fish for expenditures that contribute to somatic growth during the previous few weeks and for upstream migration during spawning.

Generally, fishes collected during fall had greater condition factors. Fish condition and time of spawning are important predictors for survival and for the ability of young fish to overwinter (Bennet and Janz, 2007; Engelhard and Helno, 2006). Goldeye move from the Peace-Athabasca Delta to overwinter in three areas: the lower Peace River; the upper SR; and even as far as the Slave River Delta (Nelson and Paetz, 1992). The Northern pike is a Holarctic species arriving near spawning grounds under ice and spawning in early spring (Scott and Crossman, 1975). Lake whitefish are bottom feeders that spawn in the fall. They start to migrate upstream into the AR from the Peace/Athabasca Delta in late August and early September. Spawning likely

occurs from mid-October to early November in the Mountain and Cascade Rapids area, near Fort McMurray. During spring, small numbers of lake whitefish move into the AR and its tributaries (especially the Steepbank and MacKay Rivers) to feed. These fish might be moving upstream from Lake Athabasca (Mill et al. 1997). Burbot enter the SR to spawn during winter or early spring, peaking in early February (Nelson and Paetz, 1992). For this study, burbot were collected in summer and fall 2011, while in 2015, burbot were collected during winter (February). Walleye are spawn in early spring and move into the rivers as soon as the ice is gone, usually ending by mid-May. Migration starts by mid-August and proceeds up the AR into the MacKay River to spawn (Scott and Crossman, 1975).

A relatively uniform incidence of anomalies was observed in fishes from the Athabasca and Slave Rivers. While relatively great frequencies of abnormalities appeared in fish collected near Fort McMurray, Fort McKay and Fort Chipewyan, this was observed only during 2011 and 2012. The 1901 fish collected over the length of the river system exhibited a relatively small overall incidence of anomalies. For most species, anomalies occurred in less than 5 percent of individuals collected. It should be remembered that assessment of anomalies included a relatively extensive variety of lesions and may have captured healed or healing wounds as well as true 'lesions' with an ecotoxicological origin. However, our main goal was to compare the relative incidence of these anomalies close to and distant from the AOSR.

Interpretation of effects of stressors such as chemical toxicants, on condition indices of fishes is often confounded by other factors including habitat quality (e.g., eutrophication) and food availability (e.g., impaired prey communities) (Bennett and Janz, 2007; Gauthier et al., 2009; Pyle et al., 2008). Trophic structures of the Athabasca and Slave Rivers might indirectly contribute to the temporal resurgence of condition factors of walleye, burbot, whitefish, and northern pike. Some

of their prey, such as chironomids and amphipods, have been increasing in numbers during recent years (RAMP, 2012). For example, the main diet of whitefish consists of bottom-dwelling invertebrates such as chironomids and amphipods, although they occasionally eat fish (Nelson and Paetz, 1992). Adults feed on fish while the young feed on immature aquatic insects (Scott and Crossman, 1975). Increases in condition factors of walleye, burbot, whitefish and jackfish from 2013 to 2015 could also be the result of decreased competition for food due to smaller population size (Schwalb et al., 2015). It should also be noted that 2011-2012 was a relatively low flow period in the AR/SR system and that similar resurgence of CF has also been noted in small bodied fish (Mark McMaster personal communication).

Complications from low flow conditions, especially in late fall and winter, can affect health of fishes. Previous studies on hydrological variability and trends in the AR headwaters have reported a decline by about 0.2% over the 20^{th} century (Rood et al., 2008) and downstream, near Fort McMurray, a decline of 33.3% from May to August from 1970 to 2003 (Schindler and Donahue, 2006). As of 2007, the 6 operating facilities extracting bitumen along the lower AR cumulatively withdrew 415 million m^3 of water per year (AMEC, 2007, cited in Schindler et al., 2007). It has been reported that a 7.22 km^3 reduction in total streamflow input into Lake Athabasca has occurred, which has resulted in a 0.87 m decline in the level of Lake Athabasca between 1960 and 2010. Similarly, construction of the W. A. C. Bennet Dam on the headwaters of the Peace River regulates the magnitude and frequency of flow alterations in the SR (Prowse et al., 2006). In addition to the dam, SR water quantity and quality are affected by the Peace River and the AR via Lake Athabasca and the Peace Athabasca Delta.

In addition to anthropogenic withdrawals, climatic variability has been reported in the basin. Average precipitation in the region has significantly decreased (Squires et al., 2010;

Timoney, 2009). Mean ambient air temperature in the Athabasca basin has significantly increased since 1966 by about 1.4 ^{o}C (Squires et al., 2010). The decrease in precipitation and increase in temperature are expected to decrease snowpack depth and thus magnitude of spring runoff and peak flows (Schindler and Donahue, 2006). Changes in water temperature are a potential stressor and could cause immunosuppression, increasing the incidence of abnormalities in fish (Audet and Couture, 2003; Eastwood and Couture, 2002; Gauthier et al., 2009). While differences in temperature might explain some of the observed variation between seasons, it might not be a factor for the seasonal and inter-annual variations in fish condition observed in this study.

Variability in discharge is frequently observed in the area and might be a contributing factor to condition factor in the area. Lesser flows in winter are common in the Athabasca and Slave Rivers during sampling years and peak flows are observed in June and July (Table 5.5). Discharge of the lower AR was greater in 2011 than it was in 2012 and 2010. Flow rates vary considerably from the AR through the SR. The Peace Athabasca Delta provides a dominant source of water to the SR, thereby significantly increasing discharge of the SR. The greater discharge of the SR results in dilution of dissolved chemicals (Culp et al., 2005). Fragmented monitoring of discharge and weather condition complicate understanding of long term effect on condition factor. However, it is important to know that the relative uniformity of condition factor observed across the study locations indicates that the variability in flow condition in the Athabasca and Slave Rivers does not appear to have a marked direct effect on the condition (Fig. 5.3). The liver somatic index showed higher levels in Fort Resolution, suggesting exposure to contaminants that have the potential to cause the observed increase. Different chemical pollutants including organic compounds can cause oxidative stress and damage in fish.

Table 5.4: Relationships between concentrations of PAHs in bile and muscle vs. condition factor

Location	Bile (p-value)	Parent PAHs (p-value)	Alkyl PAHs (p-value)
Fort Mcmurray	0.000 (0.067)	-0.001 (0.503)	0.002 (0.053)
Fort Mckay	0.000 (0.001)	0.002 (0.001)	0.000 (0.878
Fort Chipewyan	0.000 (0.041)	0.007 (0.051)	0.000 (0.612)
Fort Smith	0.000 (0.886)	0.001 (0.591)	0.004 (0.121)
Fort Resolution	0.000 (0.001)	-0.005 (0.163)	0.002 (0.219)

Significant values are R^2 ($p<0.001$), the values indicate a non-significant relationship.

Table 5.5: Hydrological parameters of AR (AR), Lake Athabasca and Slave River (SR) from 2010 to 2015. Parameter (PARAM) 1 = Flow rate (m^3/s) and 2 = water level (m). n.a = data not available. Data was obtained from the Water Survey of Canada's water quantity database (HYDAT) to represent the hydrological condition of the study area.

Location	Param	Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
AR below Fort	1 (M^3/S)	2010	138	133	140	336	603	918	868	610	652	571	220	145
McMurray	1 (M^3/S)	2011	131	123	128	286	1140	1790	2240	924	504	409	193	164
	1 (M^3/S)	2012	135	111	115	461	948	1490	1800	1170	669	473	244	211
	2 (M)	2012	n.a	1.921	1.971	n.a	2.619	3.213	3.556	2.921	2.227	n.a	2.704	2.567
Lake Athabasca	2 (M)	2010	208.5	208.4	208.3	208.3	208.5	208.5	208.5	208.5	208.3	208.2	208	208
at Chipewyan	2 (M)	2011	207.9	207.8	207.6	207.7	208.5	n.a	n.a	209.3	209	209	208.4	208.3
SR at Fitzgerald	1 (M^3/S)	2010	2270	2910	2220	2650	3210	3660	2790	2520	2470	2390	1860	2210
	1 (M^3/S)	2011	1940	1320	2020	2130	n.a	4780	6000	3950	3850	3130	2290	2410
	1 (M^3/S)	2012	2990	3000	2290	2300	3830	5100	5070	4120	n.a	3250	2280	2550
	1 (M^3/S)	2013	n.a	2900	2880	n.a	n.a	6000	5340	4610	n.a	n.a	2750	2160
	1 (M^3/S)	2014	2000	2970	n.a	n.a	4320	5000	3960	3430	2810	2700	1960	1920
	2 (M)	2010	4.337	4.554	3.506	3.24	3.384	3.511	3.121	2.983	2.958	2.914	2.699	3.384
	2 (M)	2011	3.61	2.97	3.19	3	n.a	3.95	4.38	3.63	3.59	3.28	3.06	3.93
	2 (M)	2012	4.36	4.35	3.69	3.25	3.67	4.06	4.06	3.70	n.a	3.34	3.11	3.88

	2 (M)	2013	n.a	4.45	3.93	n.a	n.a	4.38	4.15	3.89	n.a	n.a	3.29	4.07
	2 (M)	2014	4.41	4.68	n.a	n.a	3.92	4.03	3.64	3.42	3.13	3.08	2.93	3.68

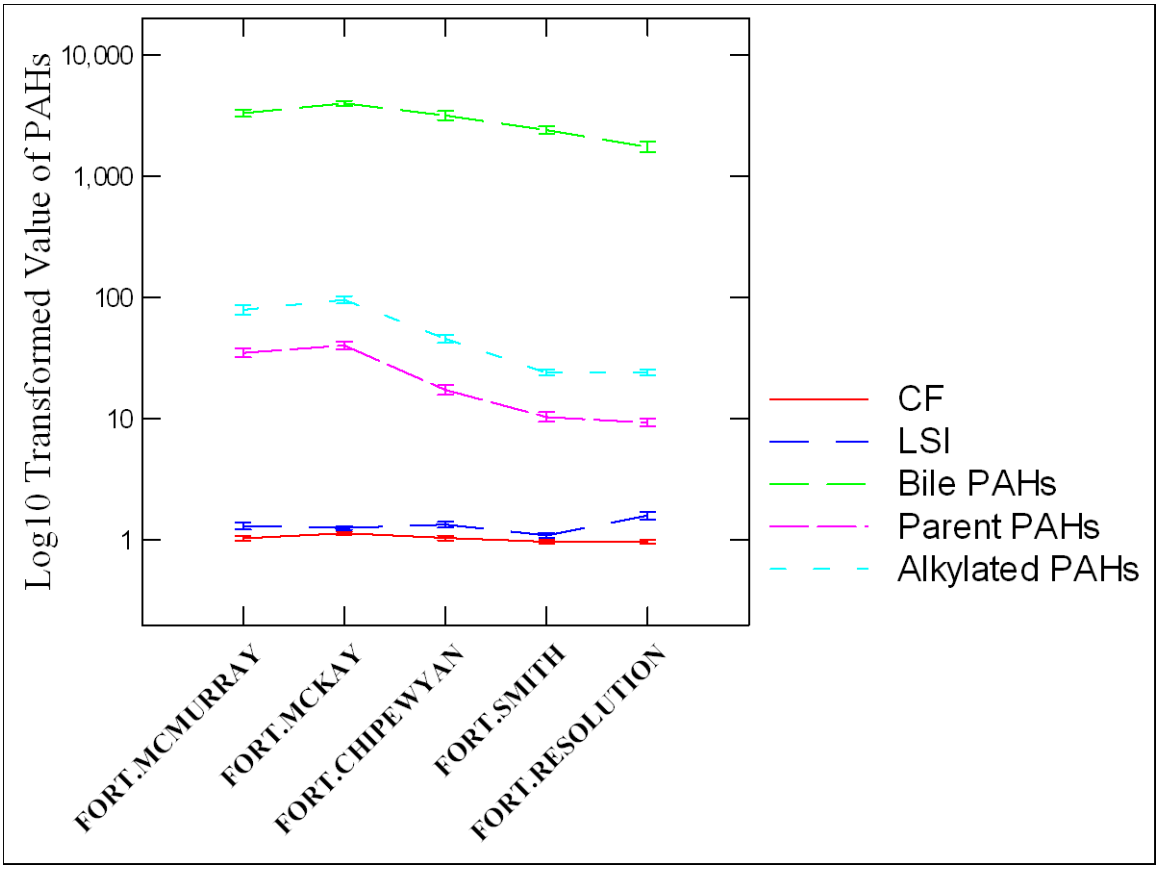


Figure 5.3: Line Graph (with standard error bars) showing the relationship between bile and muscle PAH levels and biometrics of the sampled fishes across locations (PAH values are log transformed).

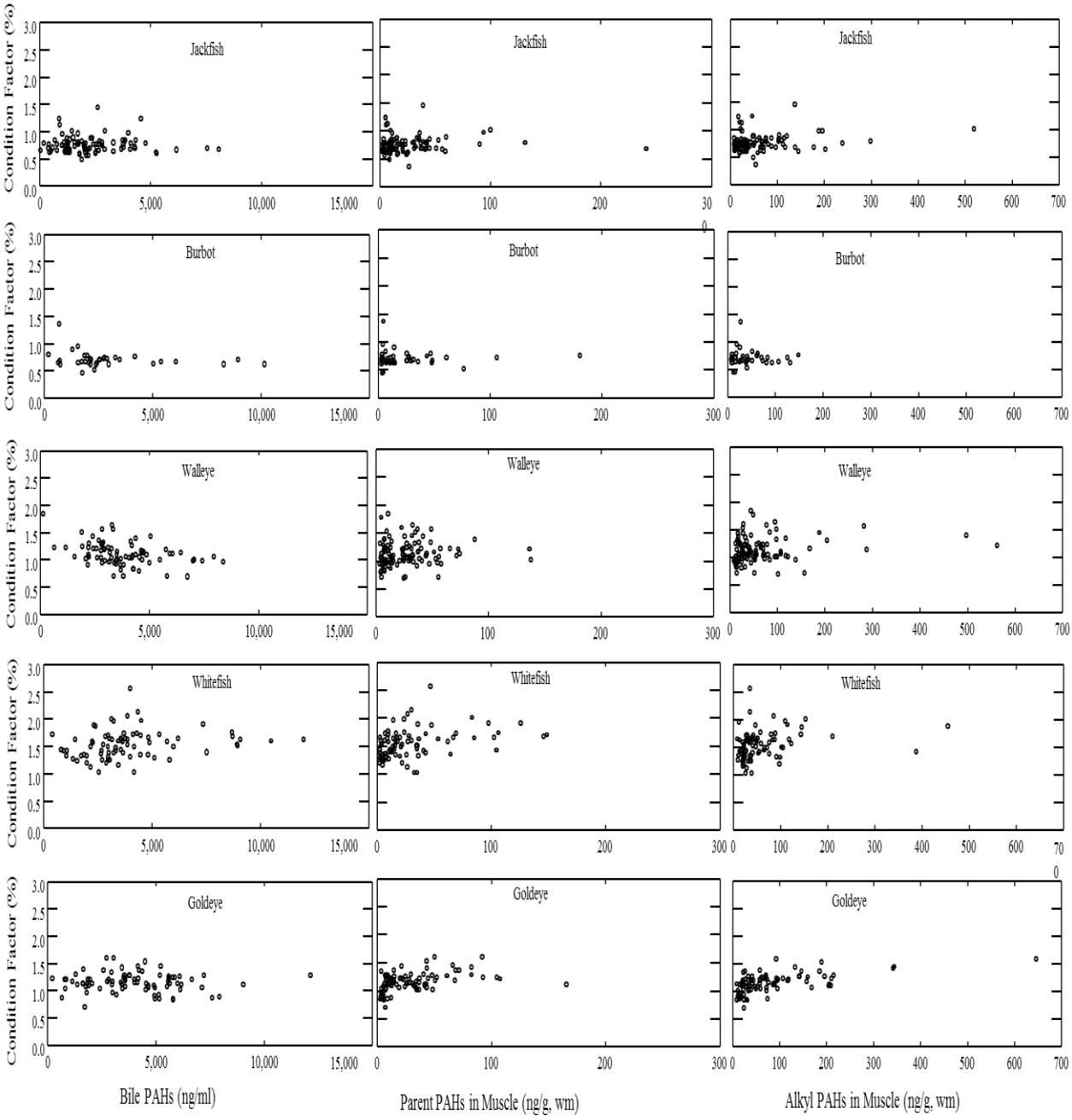


Figure 5.4: Scatter plots showing condition factor against bile, parent PAHs and alkyl PAHs concentrations, respectively.

5.0 Conclusion

This study provided an opportunity to collect detailed information about the health of selected fishes in the Athabasca and Slave Rivers. Measures of external abnormalities included turmors, lesion, scars or injuries, skin discoloration, deformities, parasites, fat deposit and coloration of internal organs. Morphometric records provide a simple, observational database that was analyzed quantitatively. Morphometric data demonstrated relatively consistent fish health in both the Athabasca and Slave Rivers. Lesions and other abnormalities were encountered in the sampled fish but their distribution was relatively uniform across locations. Analysis of the condition factor and somatic indices did not demonstrate consistent differences along the system. Overall, the health of fish does not appear to be adversely affected by current development of the oil sands. Indeed, even in the less than optimal conditions of 2011-2012 which resulted in depressed condition factors down the length of the system, oil sands development does not seem to represent an additional cumulative stressor to fish health.

Resurgence of condition factor was observed after a low in 2011. Body condition varied between seasons and years, but due to small sample size, even 2,000 fish is a relatively small sample size when monitoring temporal changes over multiple locations and multiple species. Condition can vary as a natural part of the life cycle of fish. Because of the distinct seasonal variability in condition factor, longer term data sets will be needed to capture consistent variability across species and seasons in the AR and the SR. In addition, the observed long term fluctuations in condition factor highlight the need for ongoing monitoring to determine 'normal' levels of variability against which to compare potential future impacts.

CHAPTER 6

General Discussion and Conclusions

6.1 Synopsis

It is often uncertain how lipophilic pollutants including PAHs can be taken directly by fish from the adsorbed state or whether they must first be freely dissolved into the aqueous media before they will be relatively easy for organisms to absorb. Due to the lipophilic nature of PAHs, they are easily absorbed to the particles of sediments, thereby limiting their mobility and availability to bottom dwelling fishes. It is possible that by passing sediment through their gills, fish will increase uptake of freely dissolved PAHs by altering the partitioning towards the dissolved phase. But more likely, the ingestion of contaminated sediment during feeding is the major source of PAH uptake (Walker et al., 2012).

Concentrations of PAHs were determined across spatial and temporal scales in fish from the Athabasca and Slave rivers. Fishes from the upstream portions of the AR, which were nearer to locations where oil sands are extracted and upgraded, contained greater concentrations of individual PAHs than fishes from the SR. The distribution of parent PAHs and their alkylated homologue shows naphthalene as the most prevalent compound, contributing the highest concentration to the total PAH budget, characteristic of PAH mixture generated by petrogenic pollution (Pampanin and Sydnes, 2013).

We have presented and discussed this data with our various research partners, including First Nations communities, Territorial agencies, and Federal agencies. We have also presented at scientific conferences, workshops, training courses, and seminars. The major feedback Dr. Jones and I got from members of the communities was to determine if the fish were safe to eat and the need to establish a joint monitoring program. From our findings, the fish are safe to eat. There is ongoing collaboration with communities and developing a monitoring program for fish sampling is an important component. Due in part to this project, the Slave Watershed Environmental

Effect Program (SWEEP), with Dr. Jones as the lead researcher, was put in place by the Canadian Water Network. The SWEEP project seeks to develop a community-based program that will empower communities to collect, interpret, and use a system of aquatic environmental indicators to address key concerns and priorities in the SR and Delta watershed (Dr. Jones, personal communication).

The results reported here would be valuable for establishing the status of trends and spatial distribution of PBPAHs, parent and alkylated PAHs during monitoring of the lower Peace, Athabasca and Slave basin. The results also provide baseline data on concentrations of PAHs in the probability of an increase.

6.2 Principal Findings

To address the objectives of this study, I had to optimize the analytical methods to determine PAHs in fish. The findings from my analytical study were made immediately useful in my second, third and fourth studies which set out to determine the levels of products of the bio-transformation of PAHs (PBPAHs), 16 parent PAHs in muscle, and 20 alkylated PAHs in muscle.

In Chapter 2, concentrations of 2 and 3-ringed, 4-ringed, and 5-ringed PBPAHs were present in bile of five fish species of nutritional, cultural and ecological relevance. The type of food consumed and the metabolic pathways of PAHs to their stable products of biotransformation might influence concentrations of PBPAHs in bile. Once PAHs have entered organisms, they are transported in blood and lymph, eventually moving into organs and tissues for metabolism or storage. The ingested PAHs move into cells and tissues, and are distributed between the various subcellular compartments including endoplasmic reticulum, mitochondria,

and nucleus (Walker et al. 2012). Metabolism of PAHs proceeds in Phase 1 (metabolites) and Phase 2 (endogenous molecule-conjugate). Phase 1 and phase 2 biotransformations of PAHs take place in the liver, and the gall bladder serves as storage site for PBPAHs. When PAHs move into the endoplasmic reticulum, they are converted through monooxygynase attack into more polar metabolites which partitions out of the membrane into cytosol. Either in the membrane, or more extensively in the cytosol, conjugates convert them into water-soluble PBPAHs that are readily excreted. PBPAHs move across the plasma membrane to the bile canaliculi, bile then passes into the gall bladder and is eventually released into the alimentary tract to be voided with faeces. Fish can eliminate PBPAHs across the gill, but excretion is mainly through the bile. Overall, PBPAHs were greater in lower trophic level fishes, and in those more closely associated with sediments. In particular, goldeye, consistently contained greater concentrations of all PBPAHs studied. Spatial differences were observed with increased PBPAH concentrations in bile samples of fish collected from Fort McKay Spatial increases coincided with fishes in locations proximate to oil sands operations.

Trophic level and habitat preference of selected fishes were important factors in accounting for the concentrations of PBPAH in bile of fishes. PAHs have relatively short half-lives and do not show the tendency to biomagnify. In addition, fish metabolize PAHs rapidly by monooxygenase attack. As such, they do not reach higher trophic level. However, some invertebrates of the lower trophic levels (e.g., gastropods and chironomid larvae) bioconcentrate and/or bioaccumulate PAHs, because they have poorly developed monooxygenase detoxification systems. The fishes sampled in this study are at trophic levels 2 to 4, which obtain a large proportion of their PAH residue burden directly from water and/or sediments. It is highly likely that selective predation contributed to the pollutant burden in fishes from the Athabasca and

Slave Rivers. Fish feeding lower in the food-chain are likely more exposed, considering that their invertebrate prey accumulate rather than eliminate PAHs. As such, eaten prey may contain greater PAH concentration. Goldeye and lake whitefish benthic feeding preferences make them particularly vulnerable to PAHs in sediment. The data from this study revealed greater concentrations of PAHs in first order carnivorous fishes than the largely piscivorous fishes including northern pike. This is consistent with expectations considering the trophic levels of the species studied.

Since humans mainly eat muscle tissues, it was necessary to establish the concentration of PAHs in edible portions of fishes and extrapolate these for use in assessment of risks to health of humans. Chapter 3 focused on the study of 16 parent PAHs in edible parts of selected fish species in the Athabasca and Slave Rivers. The results showed measurable levels of parent PAHs in muscle samples across spatial and seasonal studies. The profile revealed that 2-3-ring PAHs were predominant, and 4-ring PAHs were abundant. Seasonal variations were observed. The concentration of the total parent PAHs was highest in whitefish. Previous research has reported that whitefish showed considerable variability in exposure to organic contaminants, even within small geographical areas (Braune et al., 1999). This pattern was observed in this study.

Also in Chapter 3, I used the levels of parent PAHs in muscle samples to extrapolate possible risk to humans who frequently consume fish for nutritional purposes. The evaluation for human health risks was carried out using B[a]P equivalent ($B[a]P_{eq}$) of the PAH concentrations. We report $B[a]P_{eq}$ in 425 samples of goldeye, whitefish, northern pike, walleye, and burbot. The hazard ratios (HRs) were all less than 1.0, while the average, lifetime risk of additional cancers (LCR) for humans who consumed fish were deemed to be within an ‘acceptable’ range of risk (i.e., less than 10^{-6}). Comparison of fish PAH levels to human health guidelines and calculated

risk assessments indicate that PAH concentrations pose minimal health. Furthermore, a range of ultra conservative approximations of fish consumption rates (Table 3.2a), representative body masses (Table 3.2b) and the greatest observed concentration for each species (Table 3.8) were used to monitor the potential risk to fish consumers in the sampled locations. The range of possible data (from low to high values) was spread enough to include any known assessment in literature and government agencies of how much fish different groups in Canada (including Aboriginal communities) consume. The contamination with PAHs detected in the various fishes of the Athabasca/Slave Rivers did not pose a health risk to human consumers in the area. From these results, it is unlikely that PAHs derived from fishes in the Athabasca and Slave Rivers would cause adverse effects to human consumers in the area. The fresh fish samples from the Athabasca and Slave Rivers are probably a minor dietary source of PAHs. Considering our results, human consumers need not worry about the levels of PAHs in fishes from the study area. However, the carcinogenic and mutagenic effects of PAHs cannot be ignored (Vendrame et al., 2001). PAH with four or more condensed benzene rings are often mutagenic and/or carcinogenic (Johnson et al., 2004). In Fort Chipewyan, where concentrations of PAHs were less than those at Fort McMurray, we observed a greater carcinogenic potential than that of Fort McMurray (Fig. 3.4). This is because of greater concentrations of PAHs with larger TEF values, such as, benzo(a)anthracene, benzo(b)fluoranthene, and benzo(k)fluoranthene. There might be a potential human health risk if residents in these locations are fishing and consuming fishes that contain greater concentration of PAHs with larger TEF values. It is important to note that comparison of fish PAH concentrations in fishes from Fort Chipewyan in particular to human health guidelines and calculated risk assessments indicates that PAH concentrations pose no likely risk to human consumers. As a precautionary measure however, consumers in Fort Chipewyan in particular

should limit the serving sizes they actually eat to the maximum values used in this studies, i.e., children under 12, teens under 20, adults under 65 and seniors above 64 should limit daily fish consumption to 220, 350 g, 800 g and 700 g, respectively, because consuming larger portions could increase risk. These values were calculated from the MRLs that are reference values to evaluate the toxicity of PAHs based on acute (1-14 days), intermediate (14-365 days), and chronic (365 days and longer) oral exposures.

A key life history strategy for many fish species is extensive migration. During this study, differences in tissue PAH concentrations in fish of the same species were observed within the same location. Many biophysical factors lead to variability in PAH concentrations in fishes including ecology of the fish, the nature of the matrix which the PAH is in or bound to, the species taking it up, the temperature, pH and oxygen content of the ambient water (Simonin et al., 2008; Walker et al., 2012). Knowledge of fish movement is important for estimating the exposure of fish to contaminant sources. Fish migrating through polluted environment are exposed directly to pollutants dissolved or suspended in the water column, and in biota and sediments (Evans, 2000; Borga et al., 2004). Migrating species from downstream may also provide a means of delivering contaminants to other upstream species (Borga et al., 2004). Whitefish and goldeye are known to migrate long distances for spawning and foraging (Scott Crossman, 1979). Goldeye move widely within the northern Athabasca and Slave Rivers. Regular seasonal movements of several hundred kilometers are common in the mainstem of the rivers (Mill et al., 1997). High catches of goldeye are found in the Athabasca watershed, such as in the Clearwater, Christina, and MacKay rivers where they spend the summer feeding (Nelson and Paetz, 1992), an area reported to contain high levels of ambient PAHs (Akre et al., 2004). Whitefish move out of the AR in late October and early November after spawning. Some make

rapid migration downstream to Lake Athabasca and the Peace/Athabasca Delta soon after spawning. Others may overwinter in the AR. In addition to migration, size, length, and (or) age are important predictors of the levels of contaminants in fishes, with contaminant concentrations increasing with one or more of these variables (Kidd et al., 1998). It is possible that exposure of the fishes of the Athabasca basin to organic contaminants is likely at different concentration because of their different stages of development.

Whitefish represent a migratory species that may be exposed to different sources of contaminants than northern pike, which rarely travel significant distances even for spawning and as such, are good indicators for spatial comparison (Braune et al., 1999). Most northern pike do not make extensive movements. Since many northern pike do not move far during the year, point source pollution may have a larger effect on them than on more migratory species (Mill et al., 1997). Hence, northern pike served as good indicator for spatial comparison. The spatial distribution of parent PAHs varied significantly at different sampling locations with the greatest concentration in fishes from Fort McKay.

To identify the potential source(s) of PAHs, a detailed analytical study of alkylated PAHs in fishes using gas chromatography/mass spectrometry (GC/MS) was undertaken in Chapter 4. The study showed measurable levels of ∑alkylated PAHs. The degree of alkylation was observed in ∑2-ring (Naphthalenes), ∑3-ring (Fluorenes and Phenanthrene/ Anthracene), and ∑4-ring (Fluoranthenes and Chrysenes/ Benz(a)anthracenes) PAHs. The degree of alkylation was most evident in Fort McKay, followed by Fort McMurray. The PAH concentrations rapidly decline downstream to values typical of remote pristine areas (Headley et al., 2001). The distribution of 2 and 3-ring PAHs were generally bell shaped, indicative of petrogenic sources. The presence of naphthalene in environmental samples is generally associated with un-weathered petroleum that

contains relatively fresh oil (Headley et al., 2001). Naphthalene was the most prevalent compound, contributing the highest concentration to the total PAH budget, characteristic of PAH mixtures generated by petrogenic pollution (Pampanin and Sydnes, 2013). Source identification ratios indicated that fishes in the Athabasca and Slave rivers received PAHs predominantly from petrogenic sources, likely from natural oil sands deposits and development. The similarity of the distribution in the five locations suggests that the PAHs investigated are all from the same source. Although there are no visible oil sands deposit in Fort Chipewyan, Fort Smith and Fort Resolution, it is possible that river flow downstream bring in fresh organic material. Fish migrating from the McMurray formation area could also be a possible source of the petrogenic PAHs. Minor differences observed may reflect variability due to metabolism in the fishes or variability due to weathering.

Chapter 5 focused on the health status of fish from the Athabasca and Slave Rivers. Because PAHs are metabolized in the gut, released PBPAHs, and sometimes parent PAHs, may be reabsorbed into the bloodstream by passive diffusion and returned to the liver for reconjugation and repeated cycle. This process is known as enterohepatic circulation. Due to enterohepatic circulation, some of these metabolites within the organism, especially metabolites of benzo(a)pyrene have more time to exert adverse toxic actions, with the potential of causing tumors and lesions, before they are finally eliminated (Angerer et al., 1997). Lesions and other abnormalities were encountered in the sampled fish but their distributions were relatively low and uniform. Morphometric data demonstrated relatively consistent fish health in both the Athabasca and Slave rivers. Analysis of condition factor and somatic indices also did not demonstrate consistent impacts along the river system. Overall, the health of fish does not appear to be adversely affected by current levels of development of the oil sands.

PAHs may locate where they cannot interact with their sites of action and are not subject to metabolism. Of particular importance are lipophilic substrates, especially fat droplets, lipoprotein complexes and cell membranes that lack sites of action or enzymes that can metabolize PAHs. In fish, almost all of the PAHs are metabolized and eliminated but those in the fat have limited or no metabolic activity. Adaptations to the long winters in the Athabasca basin include increased lipid reserves, providing the fish with means of surviving during period of low food abundance and for reproduction during the late winter or early spring (Borga et al., 2004, Larsson et al., 1992). Reproduction requires energy investment by the mother, a large percentage of which is in the form of lipids. This provides a vehicle for the elimination of highly lipophilic chemicals (Borga et al., 2004). The sampled individuals for this study were in pre-spawning condition, spawning, and post-spawning condition. Walleye and northern pike for example spawn in the spring or early summer, while whitefish spawn in the fall or early winter. Burbot spawn in the river during the winter. Lipophilic compounds might be transported with lipids into eggs and subsequently into developing embryos (Walker et al., 2012). The rapid mobilization of fat depots will bring prompt release of stored pollutants into the bloodstream, and the pollutants will find their way to the sites of action and metabolism. In addition, greater exposure to PAHs increases the activity of metabolic enzymes that can metabolize greater concentrations of PAHs.

It is evident from the foregoing that fish efficiently metabolize PAHs, and eliminate them from their bodies into the bile for excretion. The data of parent PAHs in muscle samples and PBPAHs in bile of northern pike (Fig 6.1) demonstrate that bile sample can be used as warning system for muscle PAHs. It is important to note that parent PAHs can be extensively biotransformed into more toxic metabolites (Varanasi and Stein, 1991). Consequently, measurement of parent PAHs in fish tissues might not provide an adequate assessment of PAH

exposure (Meador, 2003). However, it should be noted that SFS method could determine all the fused ringed aromatics including heterocyclics which were not measured here e.g., dibenzothiophenes, azareenes, etc. The semi-quantification of PAH metabolites in bile using Synchronous Fluorescence Spectroscopy is suitable for environmental monitoring of pollution in the Athabasca and Slave Rivers either in the case of pollution due to oilsands operations or natural sediment contamination. Screening of bile is rapid and cost-effective and it allows priority selection of a subset of samples for detailed GC/MS analysis. This provides quantitative confirmation about individual contaminants. Biliary PBPAHs have been used as a particularly efficient biomarker of exposure in fish exposed to PAHs in their environment (Vuorinen et al., 2006; Kamman, 2007; Tairova et al., 2012), including oil spills (Budzinski et al., 2004; Kreitsberg et al., 2010).

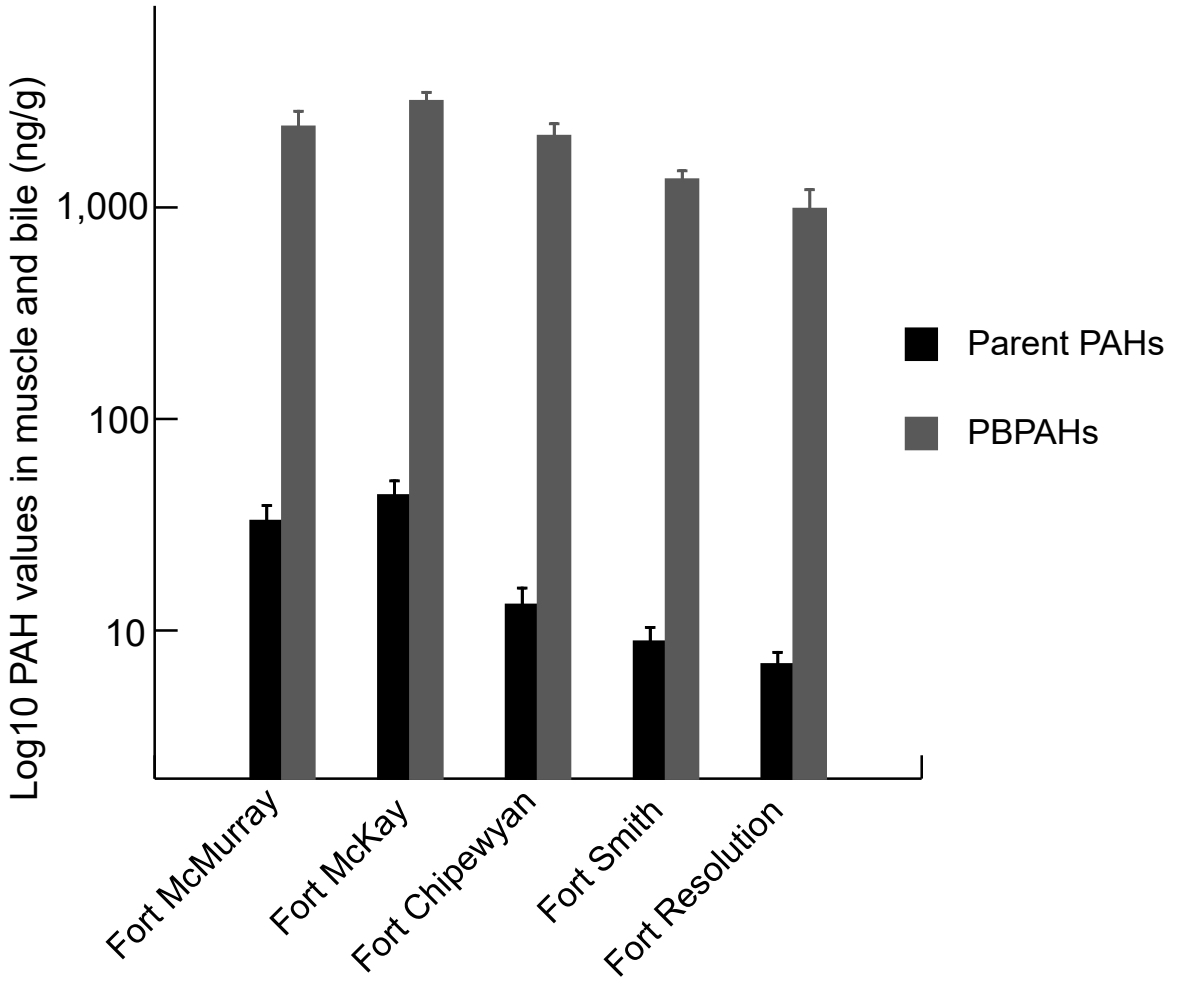


Fig 6.1: Comparison of levels of parent PAHs in muscle and products of biotransformation of PAHs (PBPAHs) in bile of northern pike.

6.3 Ongoing Research

Several areas of research are ongoing based on the results of this project. The aquatic toxicity of OSPW, its fractions, and how to distinguish between natural and anthropogenic inputs of PAHs are topics of considerable current research. The chemodynamic behavior of thallium contamination in fishes in the area is also ongoing. While a stronger regulation of the Alberta oil sands operations is necessary, emphasis should be placed on science based monitoring in the Athabasca and Slave rivers. Although the capability of fish to eliminate PAHs has been demonstrated (Dû-Lacoste et al., 2013), given the rate of expansion of the oilsand, it is possible that the concentration of PAHs in the tissues of fishes from the Athabasca and Slave Rivers might increase from the current tissue levels.

PAH concentrations in fishes from the sampled locations are widely variable. It can contain fishes with low concentration of PAHs to concentration that raise concern about ecological and human health risks. Ongoing monitoring will be important for long-term human health risk assessment. It would be difficult for First Nations community to independently monitor fishes contaminated with PAHs other than those we sampled, because sampling fish for PAHs is cost, time and logistically prohibitive. Additional routes of exposure to PAHs such as soil and air need to be considered for further studies. Also, additive effects of other chemical pollutants in the Athabasca and Slave Rivers to human health need scientific attention. It is desirable therefore that a monitoring program in water, sediments, and biota be in place and extend to the entire Athabasca basin to detect the presence of PAHs and mitigate their potential human and ecological effects. Environment Canada has a limited monitoring program in parts of the AR. Continued monitoring should focus on looking for hot spots and clean areas that can be used as reference locations. Lake sediment and fish profile analysis are needed in areas not close to tar

sands operations to capture natural contamination especially during the high flood season to give a clue of 'natural' contamination. Current ground and surface water pollution levels should be measured over time to establish the relative temporal analysis; and source of future environmental contamination including the levels of PAHs and heavy metals. There is also the need to extend this research to measure PAHs in macrobenthic invertebrate fauna. These results will help elucidate the levels of PAHs in the lower food chain in the Athabasca and Slave Rivers, and help explain the potential impact that has on fish exposures to PAHs.

The potential health risks posed by PAHs from fish consumption need to be balanced with the proven benefits of the consumption of essential omega-3, unsaturated fatty acids and minerals in fish. Self-caught fish make up large part of fish diets in the study areas. The results show that PAH concentration in fishes from the Athabasca and Slave rivers are unlikely to cause adverse health effects in human populations in Fort McMurray, Fort McKay, Fort Chipewan, Fort Smith and Fort Resolution. Overall, despite the limitation that are associated with risk analysis, the assessment undertaken indicates that the fish are safe to eat. The important role fish play in local cultures cannot be ignored. Fish provide omega-3, unsaturated fatty acids that reduce incidences of heart disease, stroke, cholesterol levels, and preterm delivery.

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Appendix A: Supplementary Information for Chapter 2

Appendix 1: Mean (± SD) values for parameters, including: length (cm) mass (g),and liver-somatic index (LSI) of fishes collected at Fort Resolution, Fort Smith, Fort Chipewyan, Fort McKay, and Fort McMurray in 2011-2012 in A) Summer, B) Fall, C) Spring. Number of individual fish sampled indicated in brackets (n). n.a = no specimen available this location/season. F= Fort.

1a) Summer

Fish Species		Fort Mcmurray	Fort Mckay	Fort Chipewyan	Fort Smith	Fort Resolution
Burbot	Length	41 ±3.4 (3)	n.a	42 ± 3.4 (2)	50 ± 9.2 (5)	62 ± 4.4 (10)
	Mass	420 ± 104(3)	n.a	693 ± 104 (2)	577 ± 320 (5)	1591 ± 341 (10)
	LSI	6.9 ± 1.5 (3)	n.a	5.1 ± 1.5(2)	2.0 ± 0.2 (5)	13 ± 21 (10)
Goldeye	Length	35 ± 4.5 (10)	38 ± 2.7 (10)	37 ± 1.1 (10)	29 ± 3.5 (10)	38 ± 1.8 (2)
	Mass	489 ± 154 (10)	685 ± 140 (10)	573 ± 55 (10)	221 ± 95 (10)	646 ± 153 (2)
	LSI	1.2 ± 0.3 (10)	1.5 ± 0.2 (10)	1.2 ± 0.3 (10)	0.7 ± 0.2 (10)	1.1 ± 0.1 (2)
Jackfish	Length (cm)	61 ± 22 (10)	62 ± 10 (10)	66 ± 5.1 (10)	68 ± 5.5 (10)	64 ± 4.2 (10)
	Mass (g)	1610 ± 1369 (10)	1938 ± 1172 (10)	2178 ± 1102(10)	2457 ± 981 (10)	1976 ± 1276 (10)
	LSI	1.4 ± 0.7 (10)	1.8 ± 0.4 (10)	0.8 ± 0.3 (10)	1.4 ± 0.6 (10)	3.3 ± 4.8 (10)
Walleye	Length	5.8 ± 10 (10)	45 ± 13 (10)	51 ± 3.4 (10)	40 ± 7.6 (10)	n.a
	Mass	1347 ± 646 (10)	1003 ± 566 (10)	1365 ± 247(10)	644 ± 364 (10)	n.a
	LSI	1.1 ± 0.3 (10)	1.0 ± 0.3 (10)	1.1 ± 0.4 (10)	0.8 ± 0.2 (10)	n.a
Whitefish	Length (cm)	n.a	42 ± 4.2(10)	41 ± 3.4 (10)	41.1 ± 3.7 (8)	39 ± 1.9 (10)
	Mass	n.a	1281 ± 323 (10)	1177 ± 324 (10)	864 ± 145 (8)	685 ± 223 (10)
	LSI	n.a	1.0 ± 0.2 (10)	1.2 ± 0.3 (10)	0.8 ± 0.3 (8)	1.9 ± 3.2 (10)

1b) Fall

Species		Fort Mcmurray	Fort Mckay	Fort Chipewyan	Fort Smith	Fort Resolution
Burbot	Length	n.a	55 ± 0.9 (2)	59 ± 2.8 (3)	61 ± 5.1 (3)	61 ± 5.0 (10)
	Mass	n.a	1075 ± 7.1 (2)	1387 ± 74 (3)	1335 ± 158 (3)	1662 ± 404 (10)
	Lsi	n.a	2.1 ± 0.1 (2)	3.0 ± 0.4 (3)	2.9 ± 0.4 (3)	3.2 ± 1.4 (10)
Goldeye	Length	39 ± 0.0 (1)	36 ± 1.4 (10)	37 ± 2.7 (10)	36 ± 1.3 (10)	36 ± 0.9 (10)
	Mass	700 ± 0.0 (1)	537 ± 47 (10)	627 ± 95 (10)	552 ± 66 (10)	546 ± 65 (10)
	Lsi	1.4 ± 0.0 (1)	1.3 ± 0.1 (10)	1.5 ± 0.5 (10)	2.1 ± 3.1 (10)	1.3 ± 0.2 (10)
Jackfish	Length	72 ± 14 (3)	63 ± 8.8 (9)	76 ± 2.5 (10)	67 ± 8.4 (10)	69 ± 11 (10)
	Mass	3287 ± 1454(3)	2531 ± 1415 (9)	4220 ± 1157 (10)	1390 ± 522 (10)	1266 ± 538 (10)
	Lsi	1.9 ± 0.4 (3)	1.9 ± 0.3 (9)	1.7 ± 0.2 (10)	1.1 ± 0.5 (10)	1.2 ± 0.4 (10)
Walleye	Length	42 ± 11 (3)	49 ± 4.8 (10)	50 ± 2.5 (5)	49 ± 5.5 (10)	47 ± 6.9 (10)
	Mass	940 ± 588(3)	1356 ± 408 (10)	4220 ± 1157 (5)	1390 ± 522 (10)	1266 ± 538 (10)
	Lsi	1.9 ± 0.1 (3)	1.5 ± 0.5 (10)	1.7 ± 0.2 (5)	1.3 ± 0.4 (10)	2.4 ± 1.2 (10)
Whitefish	Length	42 ± 3.4 (10)	40 ± 2.2 (10)	39 ± 3.1 (10)	41 ± 1.8 (10)	44 ± 3.5 (10)
	Mass	1042 ± 235 (10)	1020 ± 150 (10)	1072 ± 200 (10)	1019 ± 125(10)	1296 ± 38 (10)
	Lsi	0.8 ± 0.1 (10)	0.8 ± 0.2 (10)	1.4 ± 0.4 (10)	0.8 ± 0.2 (10)	0.9 ± 0.2 (10)

1c) Spring

Species		Fort Mcmurray	Fort Mckay	Fort Chipewyan	Fort Smith	Fort Resolution
Burbot	Length	39 ± 2.6 (3)	N.A	N.A	38 ± 0.0 (1)	63 ± 3.3 (6)
	Mass	420 ± 87 (3)	N.A	N.A	750 ± 0.0 (1)	1623 ± 632 (6)
	LSI	5.2 ± 1.9 (3)	N.A	N.A	1.1 ± 0.0 (1)	7.5 ± 3.7 (6)
Goldeye	Length	34 ± 2.9 (10)	27 ± 5.1 (10)	35 ± 3.1 (10)	37 ± 1.9 (10)	35 ± 3.8 (10)
	Mass	524 ± 113 (10)	285 ± 186 (10)	490 ± 109 (10)	570 ± 100(10)	554 ± 166 (10)
	LSI	1.1 ± 0.2 (10)	1.4 ± 0.2 (10)	1.5 ± 0.6 (10)	1.3 ± 0.2 (10)	1.3 ± 0.2 (10)
Jackfish	Length	63 ± 9.1 (10)	60 ± 7.2 (5)	63 ± 8.0 (10)	69 ± 11 (10)	69 ± 5.8 (10)
	Mass	3389 ± 1209 (10)	1862 ± 1425 (5)	1653 ± 468. (10)	3237 ± 1508 (10)	2272 ± 1020 (10)
	LSI	1.7 ± 0.6 (10)	1.4 ± 0.5 (5)	1.2 ± 0.5 (10)	1.4 ± 0.2 (10)	2.6 ± 4.4 (10)
Walleye	Length	48 ± 6.8 (10)	44 ± 2.6 (10)	50 ± 6.6 (10)	51 ± 8.7 (10)	46 ± 13 (10)
	Mass	1740 ± 870 (10)	1092 ± 148(10)	1367 ± 398 (10)	1623 ± 771 (10)	1180 ± 712 (10)
	LSI	1.2 ± 0.4 (10)	1.2 ± 0.3 (10)	1.4 ± 0.3 (10)	1.6 ± 0.5 (10)	1.5 ± 0.4 (10)
Whitefish	Length	42 ± 2.0 (4)	38 ± 1.8 (2)	43 ± 5.8 (10)	41 ± 1.3 (5)	39 ± 2.6 (10)
	Mass	1278 ± 315 (4)	1025 ± 35(2)	1384 ± 392 (10)	990 ± 115.3 (5)	807 ± 197 (10)
	LSI	1.2 ± 0.1 (4)	1.0 ± 0.0 (2)	1.3 ± 0.2 (10)	0.9 ± 0.2 (5)	1.1 ± 0.3 (10)

Appendix B: Supplementary Information for Chapter 3

Appendix B1

Log-normal probability density functions describing daily fish consumption (g/day) for Canadian Aboriginal fish 'eaters only'. Individuals reporting no fish consumption were excluded. Values were rounded to two significant digits. Values represent arithmetic mean ± standard deviation for definition of log-normal distributions. Different values for males and females are indicated only where statistically significant differences were observed between the sexes in the data. Values represent, respectively, the arithmetic mean ± standard deviation (ARITH), the arithmetic mean and standard deviation of the log-transformed data (LN-TRANS), the geometric mean and geometric standard deviation (GEOMET) (Richardson 1997 & 2013).

Sex		Children	Teens	Adults	Senior
Females	ARITH	170±150	150±150	180±140	250±240
	LN-TRANS	4.85±0.76	4.66±0.83	4.96±0.69	5.19±0.81
	GEOMET	128±2.1	106±2.3	143±2.0	179±2.2
Males	ARITH	170±150	260±250	270±190	250±240
	LN-TRANS	4.85±0.76	5.23±0.81	5.40±0.63	5.19±0.81
	GEOMET	128±2.1	187±2.2	221±1.9	179±2.2
Sexes	ARITH	170±150	200±200	220±160	250±240
combined	LN-TRANS	4.85±0.76	4.95±0.83	5.18±0.65	5.19±0.81
	GEOMET	128±2.1	141±2.3	178±1.9	179±2.2

Appendix 2

Proposed probability density functions describing body weight (kg) in the Canadian population. In all cases, PDFs should be defined as log-normal. Values represent, respectively, the arithmetic mean ± standard deviation (ARITH), the arithmetic mean and standard deviation of the log-transformed data (LN-TRANS), the geometric mean and

Age Group	Distribution	Females	Males	Sexes Combined
Infants (0-6 Months)	Arth	-	-	8.2 ± 2.9
	Ln-Trans	-	-	2.05 ± 0.34
	Geomet	-	-	7.8 ± 1.4
Toddlers (7m-4yrs)	Arth	16.4 ± 4.5	16.5 ± 4.6	16.5 ± 4.5
	Ln-Trans	2.76 ± 0.27	2.77 ± 0.27	2.77 ± 0.27
	Geomet	15.8 ± 1.3	16.0 ± 1.3	16.0 ± 1.3
Children (5yrs-11yrs)	Arth	33.6 ± 9.3	32.2 ± 8.0	32.9 ± 8.9
	Ln-Trans	3.48 ± 0.27	3.44 ± 0.24	3.46 ± 0.27
	Geomet	32.5 ± 1.3	31.2 ± 1.3	31.8 ± 1.3
Teens (12-19 Yrs)	Arth	56.2 ± 10.2	63.1 ± 15.3	59.7 ± 13.5
	Ln-Trans	4.01 ± 0.18	4.12 ± 0.24	4.06 ± 0.22
	Geomet	55.1 ± 1.2	61.6 ± 1.3	58.0 ± 1.2
Adults (20-59 Yrs)	Arth	63.1 ± 11.9	78.8 ± 12.3	70.7 ± 14.4
	Ln-Trans	4.13 ± 0.18	4.35 ± 0.16	4.24 ± 0.20
	Geomet	62.2 ± 1.2	77.5 ± 1.2	69.4 ± 1.2
Seniors (60+ Yrs)	Arth	63.4 ± 11.6	78.9 ± 14.2	70.6 ± 15.0
	Ln-Trans	4.13 ± 0.18	4.35 ± 0.18	4.23 ± 0.21
	Geomet	62.2 ± 1.2	77.5 ± 1.2	68.7 ± 1.2
Adults (20+Yrs)	Arth	63.1 ± 11.8	78.8 ± 12.6	70.7 ± 14.5
	Ln-Trans	4.13 ± 0.19	4.35 ± 0.16	4.24 ± 0.20
	Geomet	62.2 ± 1.2	77.5 ± 1.2	69.4 ± 1.2